





# CLINICAL DIAGNOSIS

A TEXT-BOOK  
*of*  
CLINICAL MICROSCOPY AND CLINICAL CHEMISTRY  
FOR MEDICAL STUDENTS, LABORATORY  
WORKERS, AND PRACTITIONERS  
OF MEDICINE

BY  
CHARLES PHILLIPS EMERSON, A.B., M.D.

LATE RESIDENT PHYSICIAN, THE JOHNS HOPKINS HOSPITAL; AND ASSOCIATE  
IN MEDICINE, THE JOHNS HOPKINS UNIVERSITY; PROFESSOR OF  
MEDICINE, INDIANA UNIVERSITY SCHOOL OF MEDICINE

*THIRD EDITION*



PHILADELPHIA & LONDON  
J. B. LIPPINCOTT COMPANY

Copyright, 1906, by J. B. LIPPINCOTT COMPANY  
Copyright, 1908, by J. B. LIPPINCOTT COMPANY  
Copyright, 1911, by J. B. LIPPINCOTT COMPANY

*Electrotyped and printed by J. B. Lippincott Company  
The Washington Square Press, Philadelphia, U. S. A.*

To  
WILLIAM OSLER, M.D.  
IN GRATEFUL RECOGNITION  
OF THE MANY KINDNESSES  
RECEIVED BY A PUPIL AND  
ASSISTANT, THIS BOOK IS  
AFFECTIONATELY DEDICATED  
BY THE AUTHOR



## PREFACE TO THE THIRD EDITION

THE flattering reception which this book has received encourages the author to prepare this, its third, edition. It is with gratitude to those who have given the book fair trial, have helpfully indicated its many faults, and, overlooking these, have used it for the good it contains, that he now undertakes to increase its usefulness. The present volume differs from those of the former editions not merely in minor details; it is, in large measure, a new book. We believe that we can rightly claim that as a text-book it is "up to date," and yet we use this expression with caution. No text-book should be "up to date" in the same sense in which a monograph on any given subject should be, for the latter must give space to much that has merely historical interest and to all of the recent work on the subject which has any promise whatever of future value. A text-book should include only those contributions to each of its subjects the value of which is reasonably certain, and only those methods which have been well tested. This standard makes the getting out of a new edition of such a book much harder than it would be did the author strive to "mention" all work on his subject which was "recent" at the date of revision.

It is but five years since the first edition of this book appeared, yet the changes which have taken place in clinical microscopy and clinical chemistry during these few years are extensive and significant. Our progress in those divisions of medical science which are based on zoölogy and botany, as, for example, clinical zoölogy and bacteriology, has been considerable; those branches based on cytology, as the microscopy of the blood, remain about as they were ten years ago; while that great mass of work in physiological chemistry has been savagely attacked by the critics.

The reason for this last fact is not far to seek. Fifteen years ago, when clinical chemistry was literally revelling in its wealth of "new discoveries," each of delightful promise, the general chemists predicted for it a humiliating downfall. Some of them even refused to see in science any place whatever for physiological chemistry, on the ground that it rested on no sure foundation. They advised us to wait until organic chemistry should have advanced sufficiently to

furnish such a foundation. Their prophecy of failure is now being fulfilled. The results of years of work have been abandoned, and it is amusing to watch our clinical chemists retracing their steps. At present they are interested less in the metabolism of the higher nitrogenous bodies, and more in that of sodium, potassium, calcium and even of water, subjects which five years ago were beneath their attention. Humiliating as it is, we must now confess that we cannot account for fluctuations in the output of even the simpler constituents of the urine. Again, we now must make such admissions as this, that "the interesting variations from the normal" of the nitrogen output, as demonstrated in various "interesting diseases," are, in fact, not variations "from the normal" at all, but are even less than the variations which healthy men under ideal conditions of observation often show. The poor patients of some clinics are enjoying peace while the hospital chemist and his students are now eating their own test diets and studying their own metabolism, in order to find out just what the "normal" really is.

Clinical bacteriology, clinical zoölogy, clinical cytology, and clinical chemistry are merely departments (and often the most difficult branches) of broad sciences and can be understood only by one who has a general knowledge of the whole science of which the medical division is a small part. The general chemist may well wonder at the audacity of the medical man who boldly uses elaborate chemical methods in attacking problems which he, with his lifelong experience in chemistry, would not think of attempting to solve. The zoölogist and the cryptogamic botanist wonder how valuable can be the opinion of the clinician concerning a few pathogenic microorganisms if this clinician is blissfully ignorant concerning the several thousand well-known (many of them ubiquitous) microorganisms of which these few are degenerate relatives. And these scientists are right. We may discover empirical uses for their methods and get results which experience shows us are valuable, but there is always danger that we shall find ourselves deceived unless our knowledge of clinical zoölogy, clinical chemistry, etc., is balanced by a broad knowledge of, and training in, general zoölogy and general chemistry. One of the severest critics of the first edition of this book berated us for suggesting to young medical students that not all the tubercle bacilli stain red by our routine method, and that barium sulphate is not perfectly insoluble in acidified urine. It is hard to understand what profit a student will get from his work in the clinical laboratory unless he has already had a preliminary training in biology sufficient to prepare him for the disagreeable revelation concerning acid-fast bacilli, and sufficient training in chemistry to make the statement about barium sulphate an old story.

No one can be more enthusiastic over the movement toward the better preliminary education of medical students than will he who teaches the methods of the clinical laboratory, for the work of his pupils will be valuable and fruitful in direct proportion as it is based on a broad knowledge of those general sciences which form the foundation of medicine. That the workers in charge of the clinical laboratories of our hospitals may unite in insisting on better preliminary training for medical students is the earnest wish of the author.

CLIFTON SPRINGS SANITARIUM, MAY, 1911.





## PREFACE TO THE SECOND EDITION

IN the preparation of this second edition the author has attempted to raise in every possible way the efficiency of this volume as a practical work for medical practitioners and students. Fully one-half the work has been rewritten, resulting, as will be noticed, in a considerable increase in the number of pages. This enlargement permits the incorporation of much new matter throughout, including the bacteriology of the sputum and urine, etc., which it is believed will add materially to the usefulness of the volume as a working hand-book. Likewise a number of new illustrations have been included from microphotographs, the work of Drs. Herman Shapiro and T. M. Wright, and original drawings by Herman Becker and D. H. Morse. These, by reason of their artistic quality and of the subjects they represent, will add much to the value of the book.

The author hereby expresses his deep appreciation of the cordial reception given the first edition, and of the kind criticisms and helpful suggestions which have indicated lines of improvement and aided in the preparation of the present edition. Acknowledgments are due Drs. Thomas R. Boggs and Wm. M. Ford, and W. L. Moss for the valuable assistance they have rendered.



## PREFACE TO THE FIRST EDITION

---

THERE have, during the past few years, appeared so many and such excellent text-books on clinical diagnosis, the clinical examination of the blood, the urine, or the gastric contents, that to add to this number one which covered the same ground in the same way as they, would seem a thankless undertaking, as well as an unpardonable misuse of energy. It is because the present work tries to cover this same ground in a different way, and one which will, we believe, commend itself to the medical profession, that we venture to offer it for inspection; we refer to the consideration of clinical laboratory work from the clinical rather than from the laboratory point of view.

This book is based on the author's experience as physician in charge of the clinical laboratory, and instructor in medicine, of the Johns Hopkins Hospital and University. He has also had at his disposal all the clinical records of the ward cases for the seventeen years of this hospital's activity.

Our course in clinical microscopy and chemistry extends over the eight months of the student's third year; two afternoons of three hours, and one of one hour, each week; but much of the work is done out of class hours, as inspection of pages 447 and 485 will show. The subjects studied are the clinical examination of the blood, urine, sputum, stomach contents, feces, and various fluids, as ascitic, pleural, cerebrospinal, cyst contents, etc. In addition to this the student follows cases assigned him in the out-patient department. To those fitted for such work simple problems of research are given. The course is a laboratory one; specimens are provided each of the students. It is needless to say that with the eighty microscopes focussed on eighty specimens of a patient's blood, sputum, etc., the most of the interesting cells or other features will be found. The best were drawn by an artist always within call. The questions discussed in the following pages are for the most part those asked by the students during the class-work. The object of this course is not so much to impart knowledge as to raise the efficiency of the student. It is not a course in chemistry and microscopy, but in these applied to the study of a patient; not in physiology, but in pathology. With the methods of chemical and biological work, with the normal findings, they are already familiar. Chemistry, inorganic and organic, qualitative and quanti-

tative, is required for admission to the school; the normal blood they have studied in the anatomical laboratory; normal urine and gastric contents, in the laboratory of physiological chemistry. We take this knowledge for granted as a foundation for the study of pathological bloods, urines, etc., paying particular attention to the clinical significance of these findings. At the same time the students are required to practise the best methods in every-day use, not only until they understand them, but until they can accurately use them. It is the practical use of a determination or examination which is emphasized. If approximate methods will do, they are used; if accurate methods are necessary, accurate work must be done, whatever the cost in time. To use an approximate method well is far better than to employ a more exact, laborious one poorly; to do approximate work is not always easy and requires practice; to be able to do accurate work well is also required of our students. Practice, experience, an exact knowledge, first of the possibilities in a method, second, and just as important, his own accuracy in the use of that method—these it is the duty of the clinical laboratory to give a student. Above all, he should train his common sense so that, using his eyes, nose, ears, and tongue, he can get results for which another man would apply elaborate methods.

The author has been careful not to include new untried methods, for of these but a small number will last, and a text-book should contain nothing as yet not well tested by friends and foes. It is the introduction of "new methods" which renders some books even dangerous to the man who buys but one.

We do not claim that with this book alone the student can study clinical microscopy. No subject in medicine is broader or requires more reference books, for some of the hardest chemical problems will at times confront him, and to interpret the various artefacts and accidental findings of the microscope would require a vast experience in microscopy, and a knowledge of zoology, botany, and mineralogy as broad as is the realm of science. For who knows what infusoria, what diatom, desmid, or other protophyte, the ovum of what parasite, the wing of what insect, the leg of what fly, the tissue of what plant, the fibre of what meat, the seeds of what berry or fruit, may be found in sputum, stomach contents, urine, or fæces, from the food, tap-water, or the contaminations from dirty vessels, or from the dust of the air? To be wise in the points of differential chemical and microscopical diagnosis is splendid; but to recognize artefacts and extraneous matter, the stumbling-blocks in diagnosis, that is the true test of the clinical laboratory worker, and this ability is gained by wide experience alone.

The function of the clinical laboratory worker is to aid the ward worker. The findings of the former are seldom conclusive, and must be interpreted in the light of the ward findings; especially is this true now that functional diagnosis is the goal. The writer can only give to the reader who has aspirations to be a clinical chemist and microscopist the advice in substance which one of Germany's greatest clinical chemists gave him when the latter regretfully left the little Swiss laboratory which had been such a pleasant home: the clinical chemist must be first a good clinician and second a chemist; he should remember that even from the laboratory point of view his stethoscope is of more importance than his microscope, his percussion finger than his whole outfit of chemical apparatus.

In conclusion, we wish to express our indebtedness to Dr. Osler for his encouragement and aid during the progress of this work, and for his hearty co-operation in placing at our disposal the records of the medical wards; and to the assistants and students of this clinic, for whose aid I am very grateful, and who are too many to mention by name except Dr. Thomas R. Boggs, whose suggestions and criticisms have been so valuable.

I take this opportunity to thank the artists who have done much beautiful work for me—Messrs. F. S. Lockwood, Hermann Becker, Max Brödel, and Mrs. Ruth Huntington Brödel, whose excellent half-tone and pen-and-ink drawings must be recognized by the lack of her signature.

CHARLES P. EMERSON.

JOHNS HOPKINS HOSPITAL, 1906.



# CONTENTS

	PAGE
INTRODUCTION.....	xxvii

## CHAPTER I.

### THE SPUTUM.

Introduction.....	17
Amount of Sputum.....	18
Consistency of Sputum.....	19
Reaction of Sputum.....	19
Character of Sputum.....	19
Color of Sputum.....	19
Layer Formation of Sputum.....	22
Odor of Sputum.....	22
Macroscopic Constituents of Sputum.....	22
Microscopic Constituents of Sputum.....	26
Plant Parasites in Sputum.....	34
Animal Parasites in Sputum.....	43
Chemical Examination of Sputum.....	45
Sputum in Pulmonary Tuberculosis.....	46
Pneumonia.....	58
Influenza.....	64
Whooping-cough.....	66
Glanders.....	67
Asthma.....	67
Acute Bronchitis.....	71
Chronic Bronchitis.....	73
Fibrinous Bronchitis.....	76
Bronchiectasis.....	77
Gangrene of the Lung.....	79
Abscess of the Lung.....	80
Perforating Empyema.....	82
Perforating Serous Pleurisy.....	82
Œdema of the Lungs.....	82
The Albuminous Sputum of Thoracentesis.....	83
Hæmoptysis.....	84
Sputum in Hemorrhagic Infarction of the Lung.....	85
Chronic Passive Congestion of the Lung.....	85
Malignant Disease of the Lung.....	86
Mediastinal Growths.....	86
Syphilis of the Lung.....	87
Pneumoconiosis.....	87
Sputum in Diphtheria.....	87
Vincent's Angina.....	92

## CHAPTER II.

## THE URINE.

	PAGE.
The Collection and Preservation of Urine. ....	94
The Amount of Urine. ....	95
The Specific Gravity of Urine. ....	99
The Color of Urine. ....	101
The Pigments of Urine. ....	102
The Odor of Urine. ....	107
The General Appearance of Urine. ....	107
The Reaction of Urine. ....	107
The Nitrogenous Bodies of the Urine. ....	113
Nitrogen. ....	113
Urea. ....	117
Uric Acid. ....	121
Purin Bases. ....	126
Ammonia. ....	127
Creatinin. ....	130
Oxyproteinic and Alloxyproteinic Acids. ....	132
The Inorganic Acids and Bases of the Urine. ....	133
Chlorides. ....	133
Phosphates. ....	137
Sulphates. ....	142
Thiosulphuric Acid. ....	147
Hydrogen Sulphide. ....	147
Sulphocyanic Acid. ....	147
Carbonates. ....	147
Calcium and Magnesium. ....	148
Sodium and Potassium. ....	149
Iron. ....	149
Lead. ....	150
Arsenic. ....	150
The Pigments of the Urine. ....	150
Indoxyl Sulphate. ....	150
Skatoxyl Sulphate. ....	153
Indigo-Red. ....	154
Paracresol and Phenolsulphuric Acid. ....	155
Potassium Iodide. ....	155
Bile Pigments. ....	155
Bilirubin. ....	156
Biliverdin. ....	157
Hydrobilirubin. ....	157
Bilifuscin. ....	157
Biliprasin. ....	157
Cholecyanin. ....	157
Choletelin. ....	158
The Reducible Body of Stokvis. ....	158
Tests for Bile Pigments. ....	158
Melanin-Melanogen. ....	161
Rosenbach's Reaction. ....	162
Bile Acids. ....	162
Diazo Test. ....	163
Ferments in the Urine. ....	168



	PAGE
Carbohydrates and Allied Bodies in the Urine. . . . .	169
Glycosuria. . . . .	171
Levulose. . . . .	188
Lactose. . . . .	189
Pentoses. . . . .	190
Inosite. . . . .	193
Glycogen (or Erythrodestrin). . . . .	193
Animal Gum. . . . .	194
Laiose. . . . .	194
Maltose. . . . .	194
Isomaltose. . . . .	194
Melituria. . . . .	194
Acetone. . . . .	194
Diacetic Acid. . . . .	199
Oxybutyric Acid. . . . .	201
Diabetes Mellitus. . . . .	203
Diabetes Insipidus. . . . .	210
Glycuronic Acid. . . . .	211
Alkaptonuria. . . . .	212
Homogentisinic Acid. . . . .	213
Uroleucinic Acid. . . . .	213
Proteids in the Urine. . . . .	214
Albumin Tests. . . . .	214
Proteids Present. . . . .	223
Serum Albumin. . . . .	223
Serum Globulin. . . . .	224
Euglobulin, Nucleo-Albumin, Mucin, Mörner's Body. . . . .	225
Nucleohiston. . . . .	229
Fibrinogen, Fibrinoglobulin. . . . .	230
Albuminuria without Definite Renal Lesion. . . . .	230
Physiological Albuminuria. . . . .	230
Functional Albuminuria. . . . .	231
Albuminuria of the New-born. . . . .	234
Albuminuria of Women in Labor. . . . .	234
Albuminuria of Adolescence. . . . .	235
Cyclic Albuminuria. . . . .	236
Hypostatic Albuminuria. . . . .	238
Albuminuria Minima. . . . .	238
Intermittent Albuminuria. . . . .	238
Traumatic Albuminuria. . . . .	239
Febrile Albuminuria. . . . .	239
Hæmatogenous Albuminuria. . . . .	239
Nervous Form of Albuminuria. . . . .	240
Albuminuria with Definite Renal Lesions. . . . .	240
Organic Bright's Disease. . . . .	240
Hetero-Albumosuria, Bence-Jones' Body. . . . .	241
Albumosuria, Deutero-Albumosuria, Peptonuria. . . . .	243
Hæmaturia. . . . .	246
Hæmoglobinuria. . . . .	247
Methæmoglobin. . . . .	251
Hæmatoporphyrin. . . . .	252
Sediments. . . . .	253
Preservation of the Urine. . . . .	253

	PAGE
Unorganized Sediments.....	254
Urates and Uric Acid.....	254
Phosphates and Carbonates..	257
Oxalates.....	259
Sulphates.....	262
Hippuric Acid.....	262
Hetero-Albumose.....	262
Xanthin.....	262
Hæmatoidin (Bilirubin).....	262
Indigo.....	263
Melanin.....	263
Hæmoglobin.....	263
Cholesterin.....	263
Leucin.....	263
Tyrosin.....	263
Cystin.....	266
The Diamines.....	267
Scheme of Sediments.....	267
Chyluria.....	269
Lipuria.....	270
Organized Sediments.....	271
Mucous Sediment.....	271
Epithelial Cells.....	271
Casts.....	274
Epithelial.....	274
Granular.....	274
Fatty.....	275
Waxy.....	275
Hyaline, Colloid, Glassy.....	276
Blood.....	276
Hæmoglobin.....	276
Pus.....	276
Cylindroids.....	277
Combined Casts; Bacterial Gasts, Urate Casts.....	278
Pseudo-Casts.....	278
Cylindruria.....	280
Testicular Casts.....	283
Tissue Fragments.....	284
Pus-Cells.....	284
Red Blood-Corpuscles.....	285
Concretions.....	286
Urate.....	286
Oxalate.....	286
Phosphate.....	286
Carbonate.....	287
Cystin.....	287
Xanthin.....	287
Fatty.....	288
Indigo.....	288
Albumin.....	288
Table of.....	288
Bacteriology of the Urine.....	289
Technic of Obtaining Specimens.....	289

	PAGE
Bacteriology of the Urine: Bacterioscopic Examination .....	289
Bacterial Stains.....	290
Spore Staining; Flagella Staining.....	291
Cultural Method.....	293
Organisms that may be found in the Urine.....	294
<i>Bacillus Coli Communis</i> .....	294
<i>Bacillus Typhosus</i> .....	295
The Paratyphoid Group.....	295
<i>Bacillus Lactis Aerogenes</i> .....	295
<i>Bacillus Alkaligenes</i> .....	296
The <i>Proteus</i> Group.....	296
<i>Bacillus Pyocyaneus</i> .....	296
<i>Bacillus Aerogenes Capsulatus</i> .....	296
<i>Bacillus Tetani</i> .....	297
<i>Staphylococcus Pyogenes Aureus</i> .....	297
<i>Staphylococcus Pyogenes Albus</i> .....	297
<i>Staphylococcus Epidermidis Albus</i> .....	298
<i>Streptococcus Pyogenes</i> .....	298
Septicæmia.....	299
Infectious Nephritis.....	299
Acute Pyelitis.....	300
Cystitis.....	300
Bacteriuria.....	303
Infection of Urethra and External Genitals.....	303
Acute Anterior Urethritis.....	305
Posterior Urethritis.....	305
Non-specific Urethritis.....	306
Bacteriorrhœa.....	306
Prostatitis.....	307
Bacteriology of External Genitalia.....	308
<i>Bacillus Ulceris Cancrosi</i> .....	308
<i>Trepanoma Pallida</i> .....	308
<i>Spirochæta Refringens</i> .....	310
Yeasts, Moulds, Sarcinæ.....	310
Animal Parasites.....	311
Prostatic Fluid.....	312
Tripperfäden.....	314
Diseases of the Kidneys: Albuminuria.....	315
Fevers, Congestion, etc.....	316
Acute Nephritis.....	317
Subacute Nephritis.....	319
Chronic Nephritis.....	319
Uræmia.....	323
Renal Atrophy.....	324
Congenital Cystic Kidney.....	325
Suppurative Nephritis.....	325
Cancer of the Kidney.....	325
Abscess of the Kidney.....	325
Tuberculosis of the Kidney.....	325
Infarction of the Kidney.....	323
Pyelitis, Pyelonephritis.....	326
Hydronephrosis, Pyonephrosis, Uronephrosis.....	327
Renal Calculus.....	327

	PAGE
Diseases of the Kidneys: Parasitic Diseases.....	327
Functional Renal Diagnosis.....	328
Cryoscopy.....	329
Electrical Conductivity.....	335
Delayed Urea Excretion.....	336
Chloride Excretion.....	337
Dilution Test.....	337
Renal Permeability.....	338
Methylene-Blue Test.....	338
Salicylic Acid Test.....	340
Phlorizin Test.....	341
Value of Tests.....	347

## CHAPTER III.

## THE STOMACH CONTENTS.

The Vomitus.....	350
The Fasting Stomach.....	353
Test Meals.....	353
Gastric Acidity.....	355
Total Acidity.....	357
Free Acid.....	358
Hydrochloric Acid Deficit.....	359
Total Hydrochloric Acid.....	360
Value of Tests for Acidity.....	361
Physiology of Gastric Secretion.....	362
Diagnostic Value.....	362
Sahli's Desmoid Reaction.....	364
Pepsin.....	365
Fat-Splitting Ferment.....	370
Rennin.....	371
Products of Protein Digestion.....	371
Starch Digestion.....	372
Lactic Acid.....	372
Other Organic Acids.....	375
Bases of Gastric Juice.....	375
Fermentation.....	376
Microscopic Examination.....	377
Absorption Power of Stomach.....	379
Motility of Stomach.....	380
Hyperacidity.....	382
Hypersecretion.....	383
Nervous Dyspepsia.....	385
Acute Gastritis.....	386
Chronic Gastritis.....	386
Mucus.....	387
Atrophy of Mucosa.....	388
Ulcer of Stomach.....	389
Cancer of Stomach.....	391

## CHAPTER IV.

## THE INTESTINAL CONTENTS AND FÆCES.

Motility of Intestine.....	390
Pancreatic Fluid.....	399

# CONTENTS

xxi

	PAGE
Pancreatic Fluid: Trypsin.....	399
Fat-Splitting Ferment.....	399
Diastase.....	400
Test Meals.....	400
Digestive Power of Pancreatic Juice.....	401
Examination of Stools.....	402
The Constituents of Normal Stools.....	402
The Reaction of the Stools.....	403
The Frequency of the Stools.....	403
The Consistency and Form of the Stools.....	403
The Color of the Stools.....	404
Achollic Stools.....	405
Fatty Stools.....	406
Mucus in the Stools.....	409
Blood in the Stools.....	410
Pus in the Stools.....	412
Undigested Food in the Stools.....	413
Microscopy of the Stools.....	414
Macroscopic Examination of the Stools.....	415
Concretions.....	416
Intestinal Parasites.....	418
Plant Parasites.....	437
Stools in Disease.....	439
Typhoid Fever.....	439
Asiatic Cholera.....	441
Dysentery, Rectal Diarrhoea, Cancer of the Rectum.....	442
Amoebic Dysentery.....	442
Pancreatic Disease.....	443
Permanent Mounts of Small Worms.....	444
To Preserve Stools Containing Parasite Eggs.....	445
Flat Worms.....	445
Stained Specimens of Worms.....	446

## CHAPTER V.

### THE BLOOD.

Technic.....	447
Fresh Blood.....	450
Red Cells.....	450
Degenerations.....	454
Leucocytes.....	456
Hæmokonien Granules.....	458
Fat.....	459
Platelets.....	459
Fibrin Network.....	459
Counting Red Cells.....	467
Counting Leucocytes.....	472
Blood Staining.....	474
Specific Gravity of the Blood.....	483
Dried Residue.....	486
Sedimentation of the Blood.....	486
Coagulation of the Blood.....	487
Fibrin Diagnosis.....	491
Bacteriology of the Blood.....	498

	PAGE
Serum Diagnosis.....	503
Red Cells.....	505
Shape .....	505
Structure.....	506
Size.....	507
Staining Properties .....	508
Granules of Red Cells.....	510
Number of Red Cells.....	513
Physiological Variations.....	514
Drugs and Therapeutic Measures .....	517
Pathological Variations.....	517
Resistance of Red Cells.....	519
Hæmoglobin.....	520
Leucocytes.....	532
Granules.....	532
Classification of Cells.....	534
Bone-Marrow.....	541
Nucleated Reds.....	542
Origin of Red Cells.....	546
Origin of Leucocytes.....	548
Foetal Blood. ....	551
Leucocytosis.....	551
Physiological... ..	553
Inflammatory.....	555
Pseudoleucocytosis.....	560
Malignant Disease.....	560
Post-hemorrhagic.....	560
Agonal.....	561
Medicinal.....	561
Mixed Cell Leucocytosis .....	562
Mastzell Leucocytosis .....	562
Lymphocytosis.....	562
Leucopenia.....	563
Eosinophilia.....	564
Iodophilia.....	567
Blood Platelets.....	568
Reaction of the Blood.....	572
Urea in the Blood.....	575
Anæmia.....	575
Secondary.....	577
Simple Primary.....	580
Progressive Pernicious.....	580
Chlorosis.....	601
Leukæmia.....	601
Myelogenous.....	605
Lymphatic (Lymphæmia).....	611
Acute.....	614
Mixed.....	616
Pseudoleukæmia.....	617
Hodgkin's Disease.....	617
Tuberculous Adenitis.....	618
Leukanæmia.....	618
Blood in Acute Diseases.....	618

# CONTENTS

xxiii

	PAGE
Blood in Chronic Diseases.....	636
Value of Blood Examination.....	650
Malaria.....	652
Fresh Blood.....	652
Tertian.....	652
Quartan.....	655
Æstivo-Autumnal.....	657
Cycle in the Mosquito.....	659
In Stained Specimens.....	662
Trypanosomiasis.....	666
Pyroplasmosis.....	668
Filariasis.....	669
Relapsing Fever.....	671
Opsonins.....	672
The Opsonic Index.....	676
Value of the Opsonic Index.....	678
The Wassermann Reaction.....	679
Preparation of Antigen.....	683
Method of Obtaining Serum.....	683
Method of Obtaining Complement.....	684
The Hemolytic Serum.....	684
Suspension of Red Blood Cells.....	684
Titration of Hemolytic Amboceptor.....	685
Titration of the Antigen.....	685
The Noguchi Modification.....	688
Antigen.....	689
Patient's Serum.....	689
Corpuscles.....	689
Hemolytic Amboceptor.....	689
Complement.....	689
Preparation of the Antigen.....	689
Preparation of Serum to be Tested.....	690
Complement.....	690
Preparation of Hemolytic Amboceptor.....	690
Preparation of Corpuscles.....	691

## CHAPTER VI.

### VARIOUS BODY FLUIDS.

Determination of Specific Gravity.....	692
Determination of Various Proteids.....	692
Determination of Fat, etc.....	693
Cerebrospinal Fluid.....	696
Transudates and Exudates.....	702
Peritoneal Fluid.....	703
Pleural Fluid.....	703
Pericardial Fluid.....	706
Synovial Fluid.....	706
Chylous Fluids.....	706
Ovarian Cysts.....	707
Hydrocele.....	710
Spermatocele.....	710
Tophi of Gout.....	710
Urea on the Skin.....	710





# LIST OF ILLUSTRATIONS

PLATE	I. Blood-cells, Ehrlich's stain.....	506
"	II. Leucocytes, platelets, and Trypanosoma, Hastings' stain.....	536
"	III. Malaria (stained).....	652
"	IV. Parasites of tertian fever and quartan fever.....	656
"	V. Parasite of æstivo-autumnal fever.....	658

FIG.	PAGE	FIG.	PAGE
1. Spiral thread of mucus from sputum.....	23	32. Ammonium biurate crystals....	255
2. Extraneous matter common in the sputum.....	26	33. Bile-stained calcium phosphate needles.....	256
3. Epithelial cells found in sputum.....	28	34. Uric acid crystals.....	256
4. Elastic tissue from tuberculous sputum.....	30	35. Triple phosphate crystals.....	257
5. Elastic tissue from tuberculous sputum showing alveolar arrangement.....	30	36. Atypical triple phosphate crystals.....	258
6. Fatty acid crystals in sputum..	32	37. Dicalcium phosphate crystals... 258	
7. Leptothrix form in sputum.....	32	38. Calcium phosphate crystals....	259
8. Elastic tissue in sputum from food.....	33	39. Calcium carbonate dumb-bells..	260
9. Mucor mucedo.....	38	40. Calcium oxalate crystals and spheres.....	260
10. Aspergillus fumigatus.....	38	41. Calcium oxalate plates.....	260
11. Aspergillus flavus.....	38	42. Various crystals.....	262
12. Penicillium glaucum.....	41	43. Hæmatoidin, leucin, tyrosin, xanthin.....	263
13. Egg of Paragonimus westermanii	44	44. Cystin crystals.....	266
14. Bacillus tuberculosis.....	48	45. Epithelial cells from urethra ..	271
15. Fibrin cast from a case of double pneumonia.....	61	46. Epithelial cells from urine....	272
16. Bacillus influenzae.....	64	47. Epithelial cast and cells, pseudopus cast, etc.....	273
17. Curschmann's spiral.....	68	48. Coarsely and finely granular casts.....	274
18. Free central fibre from a Curschmann spiral.....	69	49. Waxy casts.....	274
19. Bacillus diphtheriæ.....	88	50. Epithelial, fatty, and pus casts.	275
20. Smear from case of Vincent's angina.....	92	51. Hyaline casts.....	277
21. Kjeldahl apparatus for nitrogen determination.....	116	52. Blood-cast.....	277
21a. Heinz modification of Hufner apparatus for urea determination.....	116	53. Cylindroids.....	278
22. Schlösing's apparatus for ammonia determination.....	129	54. Pseudo-casts.....	279
23. Folin's apparatus for ammonia and acetone determination... 130		54a Micrococcus aureus.....	298
24. Apparatus for determining the melting point of crystals.... 179		54b Streptococcus pyogenes.....	298
25. Half-shadow saccharometer.... 185		54c Spread of pus containing gonococci.....	304
26 and 27. Fields of a saccharometer	186	54d Trepanoma pallida and Spirochæte refringens.....	309
28. Iodoform crystals.....	196	55. Sediment from Echinococcus cyst.....	311
29. The horismascope.....	217	56. Fragment from Echinococcus cyst wall.....	312
30. Esbach's albuminometer.....	221	57. Schistosomum hæmatobium....	312
31. Hæmin crystals.....	250	58. Accidental urinary sediments, protophytes, etc.....	313
		58a Eggs of Eustrongylus gigas....	313
		59. Prostatitis fluid.....	313
		60. Prostate fluid.....	314
		60a Cells in prostatic fluid.....	314

FIG.	PAGE	FIG.	PAGE
61. Mucus mass full of spermatozoa .....	315	100. Diagrams of the movement of cells in the coagulometer .....	490
62. Apparatus for cryoscopy .....	331	Hess's viscosimeter .....	492
63. Strauss funnel for lactic acid test .....	373	101. Tubes for collection of blood for serum-diagnosis .....	501
64. <i>Sarcina ventriculi</i> and yeast cells .....	395	102. Tube for diluting serum .....	502
65. Fats and soaps in stools .....	407	103. Widal test, negative result .....	503
66. Fatty acid crystals .....	408	104. Widal test, positive result .....	504
67. Charcot-Leyden crystals .....	415	105. Meischer's modification of the Fleischl hæmoglobinometer .....	521
68. Pseudo-eggs in stools .....	416	106. Mixing pipette for the Miescher hæmoglobinometer .....	522
69. Cells in stools .....	416	107. Color prism for the Meischer hæmoglobinometer .....	523
70. Spines forming the "down" of fruits .....	416	108. Pipette for the Fleischl hæmoglobinometer .....	523
71. <i>Amoeba coli</i> .....	418	109. Gowers's hæmoglobinometer .....	525
72. <i>Amoeba coli</i> .....	418	110. Sahli's hæmometer .....	525
72a. <i>Trichina spiralis</i> .....	420	111. Dare's hæmoglobinometer .....	527
73. Eggs of <i>Trichocephalus dispar</i> and of <i>Ascaris lumbricoides</i> .....	420	112. Pipette of Dare's hæmoglobinometer .....	527
73a. Eggs of <i>Tyroglyphus siro</i> .....	420	113. Nucleated red cells of fetal blood .....	542
74. <i>Trichomonas vaginalis</i> .....	422	114. Blood platelets .....	560
75. <i>lamblia intestinalis</i> .....	423	115. <i>Hæmamoeba leucamiæ magna</i> et <i>parva</i> ; large granular cell of bone marrow .....	616
76. <i>Balantidium coli</i> .....	424	116. The development of the malaria parasite in the mosquito's stomach .....	660
77. <i>Oxyuris vermicularis</i> .....	426	117. Intestine of an infected mosquito with oöcysts .....	660
78. <i>Ankylostoma duodenale</i> .....	426	118. <i>Culex</i> and <i>Anopheles</i> mosquitoes .....	661
79. Caudal bursa of <i>Uncinaria americana</i> .....	427	119. Heads of mosquitoes .....	662
80. Caudal bursa of <i>Uncinaria duodenalis</i> .....	428	120. Leishman-Donovan bodies .....	668
81. Head of <i>Uncinaria americana</i> .....	428	121. <i>Filaria bancrofti</i> .....	669
82. Head of <i>Uncinaria duodenalis</i> .....	428	122. <i>Spirochæte obermeyer</i> i .....	670
83. Eggs of <i>Uncinaria duodenalis</i> .....	429	122a. Method of obtaining blood .....	674
84. Larva of <i>Uncinaria americana</i> .....	429	122b. Tubes used in serum work .....	674
85. <i>Distoma lanceolatum</i> .....	430	122c. Smear of spinal fluid of a case of epidemic cerebrospinal meningitis .....	675
86. <i>Tænia solium</i> —head, link and egg .....	431	122d. Smear of spinal fluid of a case of meningitis due to <i>Diplococcus lanceolatus</i> .....	675
87. Head of <i>Tænia saginata</i> .....	434	122e. Smear of spinal fluid of a case of meningitis due to <i>Bacillus influenzae</i> .....	702
88. Links of <i>Tænia saginata</i> .....	434	123. Cells from a pleural fluid .....	704
89. Eggs of <i>Tænia saginata</i> .....	434	124. Fatty acid crystals from an ovarian cyst .....	708
89a. Links of <i>Tænia solium</i> .....	434	125. Cholesterin crystals .....	709
90. <i>Hymenolepis nana</i> .....	435	126. Sodium biurate crystals .....	710
91. <i>Bothriocephalus latus</i> .....	436		
92a. Egg of <i>Schistosoma hæmatobium</i> .....	437		
92b. Egg of <i>Schistosoma hæmatobium</i> .....	437		
92c. <i>Bacillus bifidus</i> .....	438		
93. Fresh blood-cells .....	452		
94. Thoma-Zeiss hæmocytometer .....	459		
95. Field of ruled slide .....	463		
96. Scheme of ruled slide .....	464		
97. Arm of hæmatocrit .....	472		
98. Method of making smears .....	475		
99. Bogg's modification of Russell-Brodie coagulometer .....	490		

## INTRODUCTION

THE clinical laboratory has two special functions in the medical school,—in it the student learns the application of physical and chemical methods in the study of disease, and in it researches are conducted on the innumerable problems concerning etiology, diagnosis, and treatment. Forming an essential part of the hospital-half of a school, it should be close to the wards and so arranged as to have ample facilities for the students and for the house physicians and others doing special work. It should be in charge of a man resident in the hospital, familiar with the routine of the clinic, and in close daily touch with his chief and with the assistants. The expenses should be shared equally by the hospital and the medical school. Into the details of organization I will not enter, but the director of such a laboratory should, if possible, have assistants thoroughly trained in bacteriology, physiological methods, and physiological chemistry.

In 1896, through the kindness of two ladies, a special clinical laboratory was built for the students of the Johns Hopkins Medical School, which was enlarged two years ago when the new clinical building was erected. On each of the two floors about fifty students are accommodated and there are rooms adjacent for special workers and for the assistants. Dr. Jesse Lazear was at first in charge, and under Dr. Thayer's direction the well-known researches of Macallum and Opie and of Lazear himself on malaria were carried on. In 1900, after Dr. Lazear went to Cuba, we were fortunate enough to have Dr. Charles P. Emerson take charge of the laboratory, and to him the medical school is deeply indebted for the organization of clinical laboratory courses of the most thorough and scientific character.

In medical education the all-important problem is to give a man the knowledge he can use. In our modern system much of the training is rendered ineffective, as it has not been sufficiently prolonged to become part of a man's intellectual or bodily mechanism. A brief course of six weeks on any practical subject is almost useless and in some may be positively dangerous. When possible, an orderly sequence should be followed, so that the work of each year shall supplement that of the preceding. In the seven-year course laid down by the Johns Hopkins University a thorough laboratory training in biology, physics, and chemistry is given before the profes-

sional work begins, so that a man enters the medical school proper with a practical knowledge of scientific methods and of the use of instruments of precision. In his first year of the medical curriculum the courses in histology and physiology and in the second year those in physiology, bacteriology, physiological chemistry, and pathological histology give him an insight into the structure and functions of the body, and he becomes thoroughly familiar with the use of all instruments of precision. In the third and fourth years in the hospital side of his education, for which the previous ones have been a preparation, he must have opportunities to carry on his practical work, and these the clinical laboratory affords. A student who has been interested in the mysteries and mechanism of cardiac rhythm in the physiological course should be able to take the pulse and heart tracings of the first case of mitral disease that he meets in the out-patient department, and the means should be afforded him to pass without a jar from the normal to the abnormal,—without, indeed, appreciating that there is any difference in the method of approaching the problems involved. So too a student should be able at once to attack his first case of diabetes as a problem in carbohydrate metabolism, fully prepared by previous study to approach it on the clinical side.

If the curriculum were not so full, a student could gradually work out for himself, as the patients came under observation, every detail in the application of scientific methods to clinical study, but it is found more convenient to group them together and present in orderly sequence the subjects for study. Concurrently with the systematic instruction in the out-patient department which forms a large part of the work of the third year, a course on microscopical and chemical methods is given, and each man has his own place in the laboratory at which he may work throughout the year. This book is the outcome of the work by Dr. Emerson and his students in this course during the past five years. Not only does it represent the results of a very large number of careful observations made in the laboratory, but an analysis of many important groups of cases in the wards, so that it illustrates the experience of the medical clinic of this hospital so far as it relates to microscopical and chemical methods of diagnosis. The work will be found a comprehensive and trustworthy guide in all the details of laboratory work.

But the aim of a training such as this book implies is to send out into practice men able to give patients the benefit of modern scientific methods in the diagnosis and treatment of disease—men who *use*

the microscope, who examine sputum, and who *use* the stethoscope, and who can do the routine urine and blood work with confidence. The men to study a book of this kind are the young practitioners who are keeping up the practical knowledge obtained in the medical school, and who appreciate a small laboratory as the most valuable stock-in-trade. As a practitioner becomes more and more engaged, he can hand over to an assistant the laboratory side of the work, but it is surprising how much can be done even by the busiest of men if the *will* is there and if the methods have once been thoroughly mastered.

WILLIAM OSLER.

January 30, 1906.



# CLINICAL DIAGNOSIS

## CHAPTER I

### THE SPUTUM

**Introduction.**—The examination of the sputum is fast becoming a lost art. The discovery of a few specific organisms and the hope of finding more have had as their result the neglect of the study of fresh sputum; the many points which observers of only one generation back carefully noted are now either not looked for, or if they are, are often not seen; a rich nomenclature is forgotten, especially the Latin portion, with the exception of a few terms borrowed from the kitchen. Yet, on the whole, in following a case the careful study of the sputum in the fresh state is very important, and the student who is encouraged, even required, to do this thoroughly, will soon learn that our fathers who never saw a germ could still diagnose and follow cases with an acuity with which he usually does not credit them.

In the examination of fresh sputum the eyes and nose must be trained so that the physician may be able by simple observation to form a judgment concerning the nature of the case, its stage, or the complication which it then presents.

The variety of colors, of physical and chemical characteristics, and of structures, which the sputum may present, is bewildering. Some of these are important, more are unimportant; which are which the clinical microscopist should know. He should not mistake bacteria in chains for elastic tissue, nor tobacco for blood. For a would-be Bizzozero to have expensive pictures of starch granules drawn, confident that a newly discovered parasite will bear his name, usually means that he did not study fresh sputum with enthusiasm when he was a medical student.

**The sputum** is, strictly speaking, that substance or mass of substances which is expectorated; in a more common and more limited sense it is that which comes from the respiratory passages, from alveoli to larynx. In its wider sense, considering the variety of sources which may contribute, its importance is great; for besides those from all parts of the respiratory passages it may contain constituents from the œsophagus, nose, mouth, or, through perforation into these, from any neighboring organ.

The presence of any sputum at all is usually considered pathological, but the respiratory passages are lined with mucous membrane which may secrete mucus in such amounts that it must be expectorated. Persons living in an atmosphere laden with dust expectorate every morning the excess of mucus secreted and the dust inhaled during the preceding day, all of which has been swept during the night by the indefatigable cilia to the larynx.

This morning sputum, which is small in amount, is expectorated in lumps often as large as a cherry, is tough, elastic, gray in color from the coal-dust, and with often a translucency like boiled sago due to myelin. Microscopically, it is of streaked mucus, "the more viscid streaks arising from the goblet cells, the watery from glands" (Panizza), and arranged in lines are epithelial and pus-cells loaded with coal pigment and myelin. In addition are non-nucleated cell-like masses, probably degenerated epithelium, and pus-cells clumped together in balls, which as a rule contain no pigment.

When sputum is present in pathological amounts it is raised by coughing, unless in such amounts and with sufficient *vis a tergo* as to flow from the mouth. But there are a certain number of patients who, although there is sufficient sputum for examination, persist in swallowing it, and these must be taught to expectorate. This is particularly true of children, of persons of filthy habits, and, of course, of partially unconscious patients. The doctor is rewarded for the time spent in urging those patients who can to expectorate.

One of Dr. Osler's assistants created some amusement by assiduously sitting by the bedside of a case with suspicious lung signs begging her to expectorate. At last he got a very little sputum, but it contained tubercle bacilli, and the hospital record for early diagnosis of the pneumonic type of pulmonary tuberculosis was broken.

In some cases the swallowed sputum is obtained by washing out the stomach. In children the stools must be examined. In the case of young children the point mentioned by Findlay is valuable; the finger, covered with gauze, is put into the child's throat to stimulate coughing, and the sputum wiped out with this finger.

The patient must be carefully taught to avoid expectorating saliva, nasal and pharyngeal mucus, etc., into the cup.

**Amount.**—Some general idea of the quantity expectorated is always necessary. The accurate measurement of the twenty-four hour amount, though rarely valuable, is often an aid in following a case.

In some cases, although rare, with severe cough, the sputum is so small in amount and so viscid that there is practically none obtained. Such are cases of "dry" bronchitis, diffuse bronchitis, incipient tuberculosis, rare cases of lobar pneumonia, and of caseous pneumonia. Very much is present in certain cases of chronic bronchitis, in advanced



tuberculosis with large cavities, bronchiectasis, gangrene and œdema of the lung; in hemorrhage, perforating pleural exudate, and lung abscess the blood or pus may pour from the mouth, or even drown the patient. After too rapidly or thoroughly tapping the chest the amount of albuminous sputum may be great.

The clinical chemist engaged in metabolism experiments must remember to take an abundant sputum into account, since the amount of nitrogen thus eliminated may be even 5 per cent. of the total output.

**Consistency.**—Generally speaking, this varies inversely with the amount, except in pneumonia, in which case, although abundant, it will not drop from the inverted cup. In true bronchial asthma during the first of the attack, in acute bronchitis, and in pertussis it may be very tenacious. As a rule, this characteristic is due to mucin. The explanation in the case of pneumonic sputum with little mucin is not so easy, since the water-content is so high. It is ascribed to the nucleins present in abundance, and these in alkaline medium. On the contrary, when there is little mucus and much water, as in œdema of the lungs, or pus poured from a bronchial tree denuded of its *mucous membrane*, it is very watery.

**Reaction.**—When fresh, the sputum is alkaline in reaction. Sputum which has stood some time in the cup, or which has stagnated in the body, is usually acid.

**Character.**—*Mucoid sputum* is glairy, transparent, and tenacious. If acetic acid be added, it becomes cloudy (due to the mucin). Such sputum is seen in acute bronchitis, pertussis, and early in asthma.

A *mucopurulent sputum* is one consisting of mucus and enough pus to be macroscopically visible. A small quantity of pus gives the sputum a whitish color, a greater quantity gives a yellow or yellowish-green tint, the cause of which is in dispute. There are two varieties of mucopurulent sputum. In one the sputum consists of clear mucus in which are suspended streaks and dots of pus; in the other the mucus and pus are homogeneously mixed. In the latter the pus may give the mucus a faint white haze, or may be relatively more abundant, so abundant, indeed, that the sputum resembles pure pus.

*Purulent sputum* is said to differ from pure pus only in the tenacity due to mucus; but this distinction is artificial, since in broncho-blenorrhœa there is little normal mucous membrane left. Pure pus may constitute the sputum in ruptured empyema, abscess of the lung, rupture of an abscess of a neighboring organ through the lung, trachea, œsophagus, or nasal passages.

*Serous sputum* is colorless, and very frothy from the high percentage of albumin. It is seen in œdema of the lung, perforating serous pleurisy, and in rare cases following thoracentesis.

**Color.**—*Bloody sputum* may be almost pure blood, or gain the

name from its slight blood-staining. It is found after trauma, hemorrhagic infarction of the lung, gangrene, early in acute lobar and also caseous pneumonia, pulmonary tuberculosis, tumors of the lung, intense chronic passive congestion, and "weeping" aneurism. As a rule, the blood is mixed with mucus, hence is covered by a frothy layer. The blood may be due to diapedesis or to the rupture of a vessel. Hemorrhagic sputum is in the former case a sign of severe inflammation of the lung, but of no one disease (see page 84).

Sputa colored by the *derivatives of hæmoglobin* may be of almost any color. Formerly it was taught that varying amounts of blood could explain this variety of colors, but Traube proved that unchanged blood-cells could give only a red color or reddish tint. Blood-cells retained in the lung, either in alveoli, bronchi, or tissue, soon lose their hæmoglobin, and the various oxidation products of this can give that wide range of color seen, for instance, in a subcutaneous bruise; various shades of red, brown, green, orange, yellow, chocolate. A few cells may be found, but they are pale and swollen. The best example is the typical rusty sputum of pneumonia, the color of which is due to an unknown derivative of hæmoglobin, but the sputum may be any shade of green or yellow, red, or brown. After hemorrhage into the lung-tissue, cavities, or alveoli, and the diapedesis occurring in chronic passive congestion due, for instance, to mitral disease, there may be sufficient epithelial cells loaded with granules of changed blood pigment to give a characteristic light brown color to the sputum. In destructive processes there is sometimes sufficient hæmatoidin present to give the sputum a dirty brown color. Such is the case in gangrene, abscess, infarction, and chronic passive congestion. These crystals may literally fill the sputum.

*Bile pigments* are present in the sputum in case a liver abscess perforates through the lung or the person is jaundiced, but except in icterus the term "jaundiced" should not be used. It is granted that chemically the difference between the pigments of bile-stained and similarly appearing sputum with oxidized hæmoglobin is nil, and this may be true of hæmatoidin, but, clinically, the difference between these sputa is too important to neglect.

AS GREEN SPUTA are of such importance they should be grouped together. When a patient is jaundiced, the pure mucoid sputum of a bronchitis, for example, may be of a fine grass-green color. In such cases it is the oxidized bile pigment which gives the tint. But when no jaundice is present, exactly the same color (due to the same pigment, perhaps, but with a very different significance) is sometimes seen. This occurs in ordinary croupous pneumonia during lysis, in which case the pigment is oxidized before expectorated, a process for which there is hardly time in an ordinarily sharp attack; pneumonia

ending in abscess; and subacute caseous pneumonia. It is interesting that Traube,<sup>1</sup> who first called attention to these green sputa, gives illustrations of caseous pneumonia alone. In the five cases he cited it was an early feature in three, lasting two to five days, in two for two weeks; it did not remain green in any case till death; in one case the onset of a fresh involvement was accompanied by a return to rusty sputum. In some cases of certain green tumors, chloroma, of the lung there is green sputum; finally, certain chromogenic bacteria may explain the color.

The sputum in the various *pneumonokonioses* deserves particular mention. The most common of these is *anthracosis*, or the chronic induration of the lungs due to inhaled coal-dust. The best examples of anthracosis are seen in coal-miners, though lower grades are common among our city residents. The sputum in this condition is dirty, or even quite black, from granules of coal-dust which when inhaled are caught in the accumulating bronchial secretion, or are picked up by phagocytic cells which later appear in the sputum. Many other granules, escaping expectoration, penetrate the bronchial or alveolar mucosa and collect in the interlobular lymph channels, which they render beautifully visible. It is stated that granules thus deposited are never expectorated unless freed by a destructive pulmonary process of which their presence in the sputum after the miner has ceased to inhale this dust is a valuable sign. While such a person in a new environment may have an unpigmented sputum, although his lungs are literally black, yet some coal-miners without any symptom of tuberculosis have continued to expectorate a black sputum for years after changing their occupation (Osler). *Siderosis* is due to the long-continued inhaling of metallic dusts, and occurs among workers in iron, bronze, brass, etc. The best examples, however, are mirror polishers, whose sputum is red with ferric oxide. Those who inhale much mineral dust suffer from *chalicosis*, a disease common in certain regions, as in parts of the Rhine Valley inhabited by sandstone hewers, and popularly called "stone-cutters' phthisis," "grinders' rot," etc. These patients have contracted chests, non-tuberculous hæmoptysis recurring for years, and are susceptible to pulmonary infections, as local gangrene with pneumothorax, a frequent result. As a rule they all become tuberculous. Their sputum contains much of the mineral dust they inhale. Those who work with dry dyes, as methylene blue, have deeply colored sputum; bakers expectorate doughy masses; cotton-mill operatives expectorate cotton, while particles of tobacco and of colored foods, drinks, medicines, are often found in the sputum and deceive the unwary.

Finally, chromogenic bacteria, causing "sputum cup ward infection," may materially change the appearance of sputum, *e.g.*, *Bacillus virescens*, *B. pyocyaneus*, and many others.

<sup>1</sup> *Gesam. Beitr.*, ii. p. 699, 1871.

AIR is present in the sputum in various amounts and in bubbles of various sizes. From the size of the air-bubbles can in a general way be determined the size of the bronchi in which the sputum was formed, and the effort required to expel it. Sputum from cavities and large bronchi contains no air, and hence sinks in water. This "sputum fundum petens" was formerly given an overrated diagnostic value, since it was supposed to indicate a cavity.

**The layer formation** of sputum is of value. In certain conditions, especially bronchorrhœa, bronchiectasis, putrid bronchitis, and gangrene of the lung, the sputum is abundant, and in a tall jar will separate into three layers,—an upper of frothy mucus, a lower of morphological elements, pus, tissue shreds, detritus; and a middle of the pus serum, usually an opaque watery fluid. Often a fourth layer just under the mucus consists of the material of the sediment and hangs in long shreds down through the pus-serum.

**Odor.**—Ordinarily the sputum when fresh has almost no odor. Sputum allowed to stand, or that which has stagnated in the body, soon gains, or has when expectorated, a very positive odor; that of tuberculosis and bronchiectasis is heavy, sweet, and penetrating; that of a perforating empyema is said to resemble old cheese; that of putrid bronchitis and many cases of bronchiectasis is fetid; that of gangrene is usually the worst of all. The odor of the breath has some importance, especially in tuberculosis, for it may be fouler than the sputum in the cup, perhaps owing to the fact that the warm sputum in the body scents the air more than when cold, in which case it may be odorless. Some have claimed to have diagnosed small cavities by this sign before they could have been discovered by physical examination.

**Macroscopic Constituents.**—SMALL MASSES OF PUS are common, whose size indicates, to a certain degree, the size of the bronchi from which they arise.

FRAGMENTS OF NECROTIC TISSUE occur, sometimes large in abscess and gangrene of the lung, but small in tuberculosis in which disease large masses are rare except perhaps from the wall of a cavity around which is such active proliferation of connective tissue that the necrotic tissue is dissecting free. The great majority of the fragments are almost at the limit of gross vision. The fragments from an abscess are permeated by pus-cells, hence are yellow in color; those from other conditions are dark from changed blood, while the smaller ones are black, often from coal pigment. The recognition of even the smallest is important, since in them one has the best chance of finding elastic tissue.

If the sputum be squeezed out between two plates, these small fragments can be seen as yellowish, often pigmented threads, for the most part just on the limit of vision while some are even 2 cm. long; or

as masses from those very minute to those the size of a pea. The search is much facilitated by a small hand-lens. They are found in the greatest numbers in the nummular masses from a tuberculous cavity. Necrotic fragments of cartilage from tuberculous ulcers of larynx, trachea, or bronchi are sometimes found. Tumor fragments should be looked for.

DITTRICH'S PLUGS are bodies of considerable interest. They are sausage-shaped casts of bronchi, varying in size from very small to those the size of a bean, but the majority from that of a millet- to a mustard-seed. The smaller are of an opaque yellowish-white, the larger of a dirty gray color. If crushed between the fingers they are found to have a horrible stinking odor. Microscopically, they consist for the most part of zoogloea of bacteria, fatty acid crystals, fat droplets, and cell detritus. Few cells are contained, except in some a few leucocytes indicating perhaps that the plug is fresh. Pigment granules, fragmented red corpuscles, hæmatoidin crystals, flagellates, and a lepto-*thrix* taking a fine blue with iodine solution and not yet well studied, have been found. The fatty acid crystals of the larger plugs are long and curved, while those of the shorter are fine needles. These plugs occur in any putrid disease, especially putrid bronchitis and bronchiectasis, in which case they are especially large. How these are formed we do not know (Hoffmann). Similar plugs are derived from the crypts of the normal tonsils, and especially in case of follicular tonsillitis. These are of beech-nut shape.

CURSCHMANN'S SPIRALS are perhaps the most beautiful structures found in the sputum. They occur at some time in practically every case of true bronchial asthma, and have been reported present in acute



FIG. 1.—A spiral thread of mucus from the sputum.  $\times 5$ .

bronchitis, acute lobar pneumonia, chronic pulmonary tuberculosis, and in rare interesting cases which seem to stand between bronchial asthma and fibrinous bronchitis, in which are expectorated small fibrinous casts with a few typical spirals directly continuous with the tips of their branches. Curschmann considered the spirals due to a bronchiolitis exudativa. (For a description of these spirals, see page 68). In some

sputa coarse strands of mucus and pus may be twisted into a spiral shape (see Fig. 1).

**FIBRINOUS STRUCTURES.**—Under this head we include all structures ordinarily thus termed, although in some the presence of fibrin is rather doubtful.

The pseudomembranous casts of *diphtheria* are sometimes present in the sputum. If from the throat, larynx, or trachea, they are in unformed masses, but if from the bronchi, may form arborescent casts, from the size of which may easily be judged the extent of the process. These are whitish in color and contain many epithelial cells. In *pneumonia* casts of smaller bronchi are found very often if one takes the trouble to search for them (see page 61). These are more brownish or reddish, and contain blood and many leucocytes. The most beautiful casts occur in the *chronic idiopathic fibrinous bronchitis* (see page 76). *Acute fibrinous bronchitis* accompanies various fevers, typhoid, erysipelas, measles, smallpox, scarlet fever, acute articular rheumatism, also exophthalmic goitre, pulmonary tuberculosis, mitral disease; in the rare albuminous expectoration after thoracentesis, and after the inhalation of irritating vapors and gases, similar casts have been found. Bettmann<sup>2</sup> gives a good review of the subject.

In addition to well-formed arborescent casts occur unformed masses of similar nature, evidently also from the bronchi. These were perhaps expectorated before a definite cast could be formed.

That much of this material is fibrin is very doubtful. The tests generally applied are; the physical properties of the mass (color, toughness, etc.), the fact that it swells and clears in acetic acid (which precipitates mucin), and the rapid effervescence on the addition of hydrogen peroxide. Hirschkowitz, in one from a case of tuberculosis, found only fibrin present.

Casts formed of the *mycelium of fungi* have been found. In Osler's case the small cast consisted of the mycelium of some form of the aspergillus. Casts due to a similar parasite were expectorated for years by the case reported by Devillers and Renon.<sup>3</sup>

**LUNG STONES.**—This name is applied to almost anything having the appearance or consistency of a stone. Theoretically, they could be cartilaginous, osseous, or calcareous, but to the last alone is the term strictly applicable.

*Enchondromata* and *osteomata* of the bronchi and lungs are found at autopsy, but among Poulalion's cases (Thèse, Paris, 1891) we could find mention of none in which they were expectorated. Neither do we know of any case in which the stone has arisen in a calcified infarct, nodule of bronchopneumonia, miliary abscess, pseudotubercle of

<sup>2</sup> Am. Jour. Med. Sci., February, 1902.

<sup>3</sup> La Presse Med., 1899.



actinomycosis, cladothrix, or moulds, nor from the calcified wall or contents of a cyst or tumor, although at autopsy such concretions are found. Fränkel mentions one case in which the stone was a fragment of the bronchial cartilage which had become calcified and then dissected free, and Hoffmann one of a calcified blood-clot.

But in the vast majority of cases lung stones are calcified tuberculous material. These have been classified in two groups, bronchioliths and pneumoliths.

The *Bronchioliths* are formed by the deposit of salts in the stagnated contents of a bronchus or bronchiectatic cavity. They may be from smaller or larger bronchi. One would expect them to be arborescent, but for the most part they are irregular, jagged, from the size of a millet-seed to a bean. They may be chalky or stony hard. As a rule, they are single or at most two or three in number, but sometimes several hundreds. Poulalion suspects that these great numbers are fragments of larger stones. Some "resembling coral, finely ramified, and very hard" have been described. In one case the stone weighed 0.47 gm. and had ten or twelve branches. In Atlee's case<sup>4</sup> the stone was three-quarters of an inch long and one-quarter of an inch wide at the larger end.

*Pneumoliths* may be calcified caseous areas, which, treated as foreign bodies, ulcerate into a bronchus, or the contents of a closed cavity which become impregnated with lime salts and then set free. Another source, perhaps a common one especially in those cases in which the lung parenchyma was normal, are the calcified bronchial lymph glands. Of the pneumoliths, there are two distinct varieties,—the cretaceous, which are chalky in consistency, and the calcareous, which as a rule are small and hard, and have a rough, rounded surface. Their size varies from that of a millet-seed to a pigeon's egg.

Chemically lung stones consist of calcium and magnesium as bases with carbonic, phosphoric, and sulphuric acids, also traces of ferric oxide and other metals. Their composition varies; in some, one or another salt predominating in a large mixture, while others seem composed of but one calcium salt.<sup>5</sup> Unless the stone is branched, or in it can be found tissue structure, one cannot state definitely what its source was. Cases which expectorate any stones usually expectorate them in such numbers, even 200 and in one case 500, that the name "pseudophthisis calculosa" was formerly given to the condition. To explain these cases Hoffmann and others consider it necessary to assume a constitutional abnormality, an increased excretion of lime salts through the lungs. In these the "hæmoptysis calculosa" is often a feature of the

<sup>4</sup> Am. Jour. Med. Sci., vol. cxxii., 1901.

<sup>5</sup> See Stern, Deutsch. med. Wochenschr., 1904, No. 39; Carlyon, Brit. Med. Jour., 1890, ii. p. 1474.

“bronchial colic” accompanying the expulsion of the stone, and is due to trauma of the mucosa; while usually not abundant it may be extreme. Abscess, gangrene, or pneumothorax may result from the presence of these stones. Some concretions have a foreign body as a nucleus, a cherry-stone or a grain of wheat. In others the lung-tissue impregnated with the salts remains, and when decalcified the structure of the lung, even with some few remaining nuclei, may be seen in the sections, and the tubercle bacilli demonstrated.

Among FOREIGN BODIES expectorated may be mentioned teeth, cherry-stones, and coins.

Fragments of the wall of echinococcus cysts or the daughter cysts themselves may be expectorated.

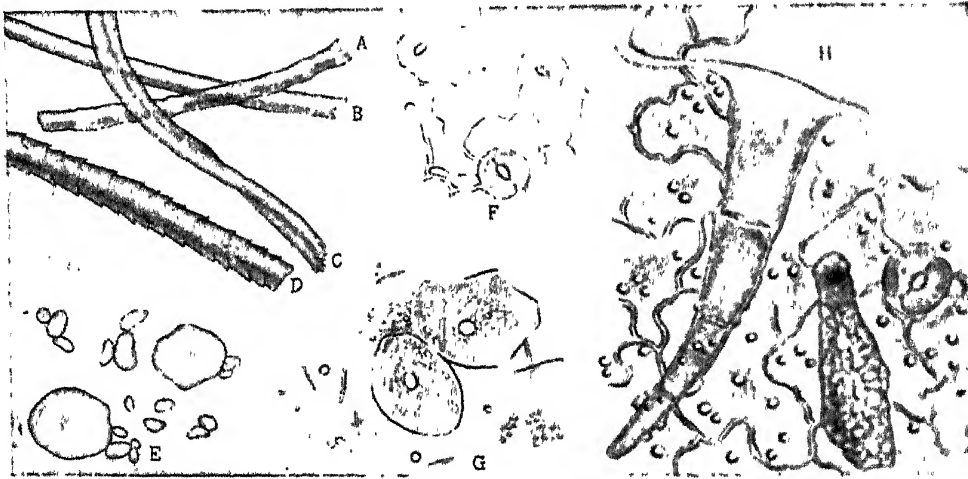


FIG. 2.—Extraneous matter common in the sputum. Threads of, A, linen; B, silk; C, cotton; D, wool; E, starch granules; F, guard cells from a lettuce leaf; G, squamous epithelium from tongue, with bacteria attached; H, tobacco, showing the surface of the leaf, the large cells stored with oil, and a spine from the surface.  $\times 200$ .

**Microscopical Examination.**—The microscopical examination of the fresh sputum is easy, valuable often, but very much neglected. The technique is of course simple. A little sputum is spread upon a plate, the base of which is half black, half white, and the interesting particles chosen and squeezed between the cover glass and the slide. Thin specimens are essential. The first point of importance is that the observer recognize at a glance the EXTRANEOUS STRUCTURES, and these are many in number. Among them may be mentioned almost any of the food-stuffs, but particularly fragments of bread, bits of orange-pulp or other fruits, drops of milk, portions of jams and preserves, the skin of fruits, portions of tobacco, etc., portions of meat in which is elastic tissue, and fragments of vegetable leaves. In addition, it is important to recognize various threads, particularly fibres of linen, cotton, wool, and silk, and small fragments of paper (see Fig. 2).



PUS-CELLS.—Thin smears of sputum may be treated like blood smears and stained with the same stains, especially the methylene blue-eosin mixtures.

The pus-cells are usually polymorphonuclear neutrophiles. They are spherical in shape, of from 7 to 10 microns in diameter. These granular cells are often filled also with fat globules or pigment granules; in some glycogen may be demonstrated. In asthma the eosinophilic cells usually predominate, and one may search long for any other form. There is a form of bronchitis which has been known as "eosinophilic bronchitis," since so many eosinophiles are present in the sputum. Hilderbrandt<sup>6</sup> holds that their presence speaks neither in favor of asthma nor against tuberculosis.

The various EPITHELIAL CELLS are important. Since these come from several sources, many forms may be expected and their origin should be recognized. *Paracment epithelium* may come from the mouth, the pharynx, and the respiratory tract as low as the vocal cords. It is a very valuable lesson for the student to scrape from the surface of the tongue by means of a cover-glass a little of the superficial epithelium and study the masses of epithelium covering the villi, to which are attached large zoogloea of bacteria. *Cylindrical epithelium* may come from the nose or bronchial tree. While the cylindrical epithelium cells from the trachea and bronchi are both goblet and ciliated in the sputum, the majority soon lose their original shape and can merely be recognized as cylindrical cells. These occur early in bronchial catarrh, and later are replaced by pus-cells. It is seldom that cells which are actually ciliated are seen in the sputum except in asthma, ulcerative processes, and recent bronchitis. In a recent case of asthma small clumps and a rather large sheet of cylindrical epithelium were found.

The ALVEOLAR EPITHELIAL CELLS it is important to study. They are present in considerable number in nearly every sputum examined, even in that of normal persons, and they may assume a large variety of forms, some of which it is very difficult to recognize. Every observer is pretty certain to be deceived once or oftener by these cells. In general, they may be said to be from four to five times the size of a leucocyte, oval, with a coarsely granular protoplasm and one or more large, oval, vesicular nuclei. They are found in normal sputa, as well as in almost every other condition. Their large number in bronchitis would indicate some intimate pathological relation between the bronchi and the alveoli. They occur in largest numbers in the sputum of patients with inflammatory processes in the lungs, especially tuberculosis. In some of the tuberculosis cases, however, although these cells may fill the alveoli they may not be found in the sputum. These cells are

\*Münch. med. Wochenschr., 1904, No. 3.

amoeboid on the warm stage, and, to judge by their appearance, are active phagocytes. Only recently has it been agreed that they are epithelial in origin.<sup>7</sup> Their contents deserve careful study. Some contain *coal pigment* (Fig. 3, *a*), that is, black granules all of which are supposed, perhaps without sufficient proof, to be particles of carbon. The origin and composition of these granules were long disputed, but ever since one single granule was found which was unquestionably a particle of charcoal, sooty air has been blamed for all black granules in the sputum. It is this pigment, the inhaled dust of the preceding day, which gives the color of dirty gray to the morning sputum of healthy men. When it is abundant the sputum is smoky or dirty green in color. Some pulmonary conditions formerly bore the name *phthisis melanotica* because of the abundance of black pigment in the sputum. There may, however, be reason to doubt whether all this pigment was carbon. The *fat globules* (Fig. 3, *i*) which some cells contain are spherical, very refractile, and glistening. *Myelin globules* are irregular in shape, often concentrically marked, only slightly refractile, and have a dull greenish or bluish tint. Some cells contain many myelin globules, others a few or but one. Many globules are very fine, while other cells are practically filled by one (Fig. 3, *h*). Myelin is said by some to be a product of the degenerated protoplasm of the cells containing it, and by others to be the normal secretion of the bronchial mucosa, droplets of which the phagocytes ingest. Cells containing myelin are numerous in the morning sputum of some healthy persons, and very abundant in the sputum of some cases of bronchitis and influenza, and of certain cases of pneumonia during resolution, especially the "desquamatory catarrhal pneumonia." The sputum in these cases resembles boiled sago, the "sago granules" consisting of masses of these cells filled with myelin droplets. *Free myelin* in large amounts is sometimes found in the sputum. It occurs in pale, non-refractive drops varying much in size and more in shape (Fig. 3, *j*). It was to these, which somewhat resemble the myelin of nerve-tissue, that Virchow gave the name "myelin drops." The term conveys no chemical significance; it is merely descriptive. Droplets bearing the same name occur in nerve-tissue, in the urine, and in the stools. Small drops of oils, fatty acids, and various neutral fats have this same appearance (Liebreich). The myelin of the sputum consists chiefly of protagon, cholesterin, and lecithin. It swells somewhat in water, is not destroyed at 100° C., is stained yellow by iodine, is stained poorly by aniline dyes, is not blackened by osmic acid, and is easily soluble in alcohol, slightly soluble in ether and in chloroform. In the morning sputum the quantity of myelin may exceed that of the mucus. To a certain degree the excre-

<sup>7</sup>See Hoffmann, Nothnagel's System, Die Krank. der Bronchien.

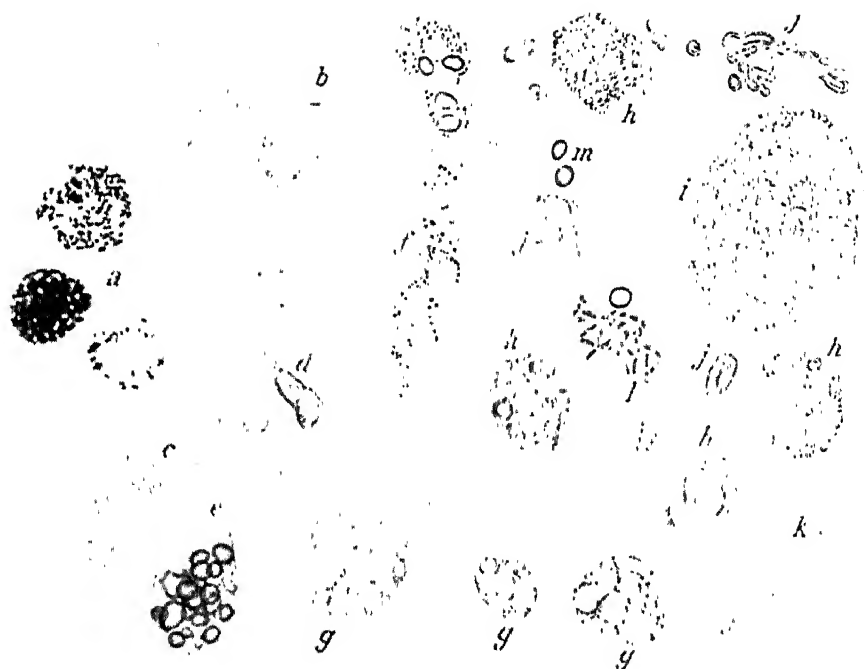


FIG. 2. Cells in the spleen: *a*, alveolar epithelium cells containing coal-dust; *b*, squamous epithelium cell; *c*, *d*, cylindrical epithelial cell; *e* and *f*, Herzfehlerzellen; *g*, cells showing a peculiar degeneration; *h*, those with myelin droplets; *i*, one full of fat droplets; *j*, free myelin; *k*, red blood-cells; *l*, bacteria; *m*, free blood pigment.  $\times 500$ .



tion of these two bodies runs parallel. Myelin globules present a variety of shapes; concentric spheres or club-shaped masses, small globules or by confluence of small globules, larger drops (see Fig. 3, *j*). On standing their number and size are greatly increased. The alveolar cells containing *derivatives of hæmoglobin* are of particular interest. The hæmoglobin, certainly derived from the red blood-cells of the sputum, may be present in amorphous granules or scales of a brownish color, or hæmatoid crystals. "Herzfehlerzellen" (see Fig. 3, *f*) is the name given to these cells filled with a golden yellow pigment, when they occur in large numbers, and over a long period of time; only then have they any diagnostic importance. The granules are sometimes small but often large. They have a translucent appearance, are not opaque, and certain cells seem to be diffusely stained. Since these granules are not opaque or deeply colored, but seem only tinged yellow, the student at first is disappointed in their appearance. In chronic passive congestion, especially that due to mitral disease, these cells may give a gross color to the sputum, the entire mass being of a rusty color; or, what is more common, they are clustered into dots and streaks of a reddish-brown color in a white mucous background. They occur also in all other conditions in which red blood-cells escape into the alveoli, namely in pneumonia, infarction of the lung, and after pulmonary hemorrhage.

THE RED BLOOD-CELLS (see Fig. 3, *k*) in the sputum are often well preserved, yet not always, as is seen by the masses of amorphous hæmoglobin and by the inclusions of the alveolar cells. They are crowded into lines and masses, allowing nothing of their shape to be seen, and are recognizable only by their color. They are sometimes squeezed out into long threads. In judging the importance of blood in the sputum, however, even macroscopically, it is well to bear in mind the numerous sources it may have had; for instance, the nose, the mouth, and the pharynx.

ELASTIC TISSUE is a most important body. Formerly its presence was of greater importance, and before the discovery of the tubercle bacillus was the best evidence of consumption. Even now it is of considerable value, since its presence indicates certainly destruction of the lung, and in some cases it is found before the tubercle bacilli, but perhaps not before they are present. The masses of elastic tissue are usually almost on the limit of vision with the unaided eye. This is particularly true in tuberculosis, in which molecular disintegration is the rule, although in some cases only single fibres may be found. The Sir Andrew Clark method is the one which Dr. Osler recommends, as by its use the various methods of destroying other tissue, such as boiling with sodium hydroxide, etc., may be dispensed with. For this two glass plates are used,—the one about fourteen and the other six

inches square. The sputum is poured on the larger plate, pressed out by the smaller. The plate should rest on a dark background. With a small hand-lens fragments of tissue may be easily selected, and then

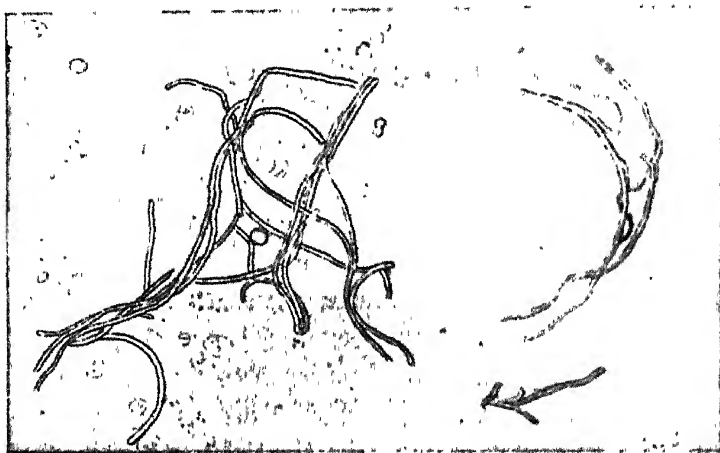


FIG. 4.—Elastic tissue from lung.  $\times 400$ .

after sliding the upper glass away from them they can be picked up with a needle. These appear as small grayish-yellow spots. In some cases it is not necessary to remove them for inspection with the higher power, since a small pocket-lens will be sufficient. Others prefer Petri's dishes, or wooden boxes with black base, or crockery plates with the base half black, half white. One must be very careful not alone to sterilize this glassware, but to wash it well in chemicals which will

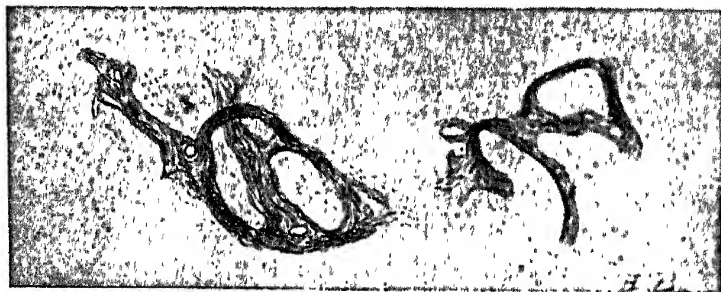


FIG. 5.—Elastic tissue from lung showing alveolar arrangement.  $\times 50$ .

destroy all organic matter (a saturated potassium bichromate solution in concentrated sulphuric acid is recommended), else there is chance that the bacilli found may not be from the sputum in question. Particles of food will confuse the beginner. Under the cover-glass small fragments of elastic tissue may be found with the low power of the microscope. Larger fragments of lung-tissue are sometimes present. When no fragments are found in this way, a search with the higher power must be made for single fibres of elastic tissue, in which case it is well to select the grayish masses of sputum from the

tuberculous cavity, or the grass-green or slightly rusty particles which are present in the sputum of subacute caseous pneumonia. The fresh specimens must be very thin and the cover well pressed down, or the fibres may be overlooked.

The elastic tissue from the lung may be present in three arrangements, depending on its source: fibres from the alveolar walls may preserve the outline of one or several alveoli (see Figs. 4, 5), and are long and branching; the fibres from the bronchial walls occur singly or in small groups, are often fragmented, and often present what Dr. Osler considers the most characteristic picture, two or three long narrow fibres clustered closely together in an elongated net-work; from the arteries may arise a distinct sheeting. Fragments containing a coarse net-work of short interwoven fibres are seen from ulcers of the larynx.

When the elastic tissue is very small in amount it is customary to destroy all other tissue by means of potassium hydrate, but if the above Clark method be carefully used this method is not necessary. Ten cc. of sputum are mixed with an equal amount of 5 to 10 per cent. KOH or NaOH; the mixture is then boiled in a porcelain dish until the mass is homogeneous. About four volumes of water are then added, the entire mass shaken up and centrifugalized. Nothing is left but the elastic tissue. The fibres, however, have lost their characteristic appearance, being now paler and swollen.

The fibres of elastic tissue (see Fig. 4) even when single should be recognized. They are characterized by their intense refractivity, their wavy outline, their sharp edges, their uniform diameter, and their curling ends. They often branch. They are insoluble in ether, potassium hydroxide, and on warming. Pressure does not cause any varicosities. Their appearance is very characteristic with the low power, perhaps more so than with the higher, although the latter should always confirm the former. In the thin specimen they will stand out as very distinct, coarse, sharp, blackish fibres. It is necessary to exclude fibrous tissue, fatty acid crystals (see Fig. 6), bacteria, and vegetable cells and fibres. The fibrous tissue fibres are very different in appearance, being present in bundles of fine wavy lines without the coarse black refractive appearance of elastic tissue. (For the fatty acid crystals, see page 33.) The chains of bacteria are very confusing, especially certain leptothrix forms (see Fig. 7). These are found in the fresh sputum, but especially that which has stood for some time. The long chains will sometimes present a beautiful interlaced net-work and sometimes simulate closely the framework of an alveolus. Under the high power, however, it will be seen that these fibres are chains of bacilli. They differ also in size and in refractivity and in the absence of the wavy outline. They are also much more crowded

in the field than is elastic tissue. Vegetable cells and fibres are much coarser and should not confuse. The elastic tissue from food is often

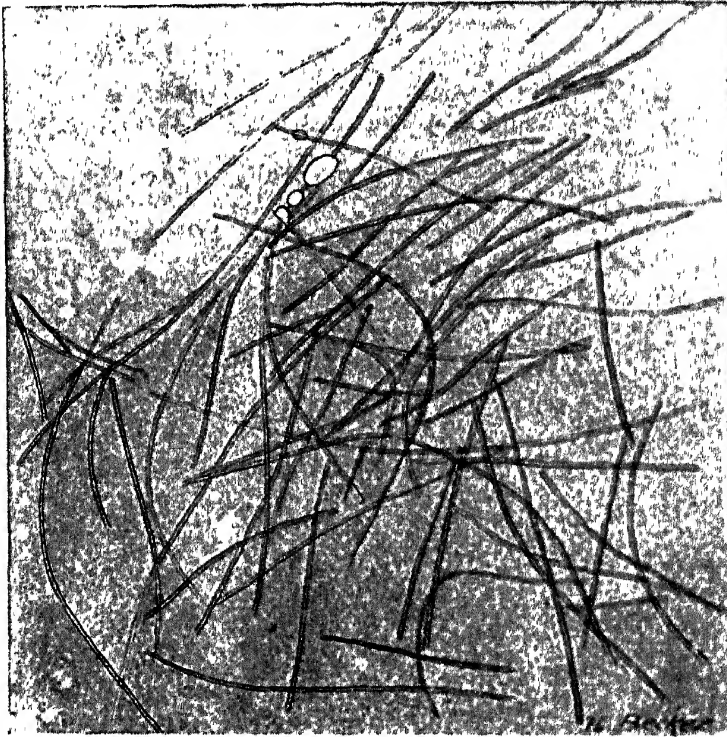


FIG. 6.—Fatty acid crystals resembling elastic tissue in the sputum of a case of bronchiectasis.  $\times 400$ .

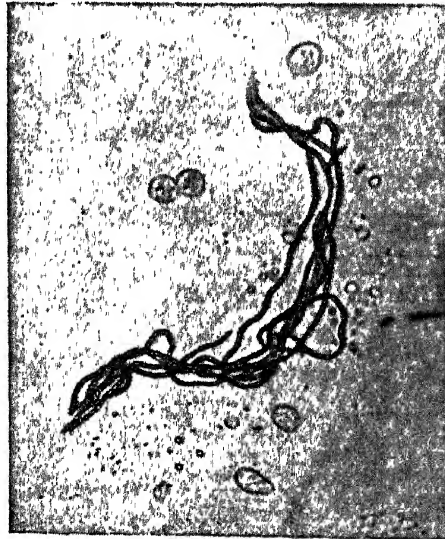


FIG. 7.—A leptothrix form in the sputum, resembling elastic tissue.  $\times 400$ .

coarser and of more irregular outline (see Fig. 8). Moulds should be easily recognized (see page 37).



The presence of elastic tissue is the surest sign of disintegration of the lung. About 90 per cent. of the cases are of tuberculosis, the rest are of gangrene, abscess, and new growth of the lung.

CRYSTALS are present only in decomposed sputum. They occur chiefly in putrid bronchitis, bronchiectasis, tuberculosis, and gangrene. *Fatty acid crystals* are found in clusters in a mass of detritus. When very long they may simulate elastic tissue (see Fig. 6), but they are usually relatively thick, with stiff curves and pointed ends. If pressure is made on the cover-glass, varicosities will result. They are soluble



FIG. 8 —Elastic tissue from saliva; origin, the food.  $\times 400$ .

in potassium hydroxide and in ether. (The specimen should be dried before ether is added.) If the slide be warmed, these crystals will disappear, and fat droplets will appear in their place. It is easy to confuse these needles with elastic tissue and streptobacilli. *Cholesterin crystals* (see page 263) are seldom found in the sputum. If they occur it is usually with fatty acid crystals. *Leucin and tyrosin* (see pages 263, 265) may be found in sputum which has decomposed in the air-passages and in the first discharge from a pulmonary abscess. The sheaves of long, black, refractive needles of tyrosin are more easily found than are the spherules of leucin. To demonstrate the latter, it is usually necessary to evaporate the sputum. *Triple phosphate crystals* (see page 257) and *calcium oxalate crystals* (see page 260)

also occur in putrid sputum. Hæmatoidin, in rhombs or in needles (see page 262), are seen in the sputum in cases of abscess of the lung, of perforating empyema, and of a liver abscess discharging through the lung, but seldom after pulmonary hemorrhage, for in the last case the extracellular hæmoglobin appears as amorphous granules.

*Charcot-Leyden crystals* (see Fig. 67) are long, narrow, diamond-shaped crystals, which resemble two very sharp pyramids with their bases together. They are colorless, but have a slight yellowish refractivity. They seem quite brittle. They vary greatly in size and are found singly, in groups, or in clusters. They are soluble in hot water, in mineral acids, and in alkalis, and they stain red with eosin. Viewed in the direction of their long axis, as when clustered, they are seen to be hexagonal on cross-section, and they therefore cannot be identical with Böttcher's spermin crystals, with which they were formerly identified. They are almost certainly derived from eosinophile leucocytes. In favor of this view is the fact that they are found only where these cells are increased, as in the sputum in asthma, in the blood in myelogenous leukæmia, and in the stools of patients with intestinal parasites. They are probably products of decomposition, for they increase in number if any specimen rich in eosinophile cells be left in a thermostat.

**Plant Parasites.**—The *bacteria* which are usually present in the sputum in so large numbers are chiefly saprophytes from the mouth, the sputum cup, or the air. These multiply very rapidly at room temperature. The chromogenic organisms are mentioned on page 21.

If the patient expectorates into a sterile cup, and the sputum be rinsed several times in sterile physiological salt solution and examined at once, then most of the organisms found will be from the lower air-passages. Among these are the host of saprophytes which are abundant in chronic bronchitis, and especially so in tuberculous and bronchiectatic cavities. These aid in the decomposition of the stagnant sputum. The pathogenic staphylococci and streptococci which also are so frequently found in these conditions are probably as a rule secondary invaders and aid in the destructive processes in progress, and also in the causation of complications and sequelæ. The specific organisms of tuberculosis, diphtheria, influenza, etc., will be described in connection with these diseases.

*MICROCOCCUS CATARRHALIS* is a common inhabitant of the respiratory passages of persons diseased or well. It is often seen in the nasal secretion and sputum of patients with common colds, and has been carefully studied in connection with epidemics of influenza. When it occurs as biscuit-shaped diplococci, it closely resembles the *Gonococcus* and *Meningococcus intracellularis*: when in chains, it is similar to *Streptococcus pyogenes*, except that the individual cocci

are larger and can be decolorized by Gram's method. It is often intracellular. It is believed by some to be identical with the meningococcus, but most recent writers (*e.g.*, Holt) consider it an organism unworthy of serious attention.

*STREPTOCOCCUS MUCOSUS CAPSULATUS* was found by Buerger<sup>8</sup> in the cerebrospinal fluid of a fatal case of acute meningitis and in the mouths of six normal persons. It is one of the group of organisms which are now held responsible for some of the epidemics of "influenza," and seems also an important cause of complications in cases of grip. It is an organism, sometimes highly virulent, in human blood nearly unsuceptible to phagocytosis. Obtained from the blood and secretions of inoculated mice, it is found to occur chiefly as round, biscuit-shaped or slightly lancet-shaped encapsulated cocci, most of them in pairs, but always a few in chains of four or even six, often irregularly arranged. The capsule is wide and easily broken. Those occurring in pairs somewhat resemble *Diplococcus lanceolatus*, but they are never so regularly and definitely lancet-shaped as are these. Their capsules are wider and do not show traces of constrictions corresponding to the spaces between the organisms so often seen in the organism of lobar pneumonia. This organism grows on most common media, but best on serum agar and glucose serum agar. On these the growth is luxurious, and the organism remains encapsulated through many subcultures. The colonies have the same watery, almost transparent, appearance as have those of *Diplococcus lanceolatus*, but they grow faster and tend to coalesce, so that the surface of the slant medium is finally covered by the growth.

*STREPTOTHRIX PSEUDO-TUBERCULOSA*.<sup>9</sup>—This streptothrix was first found in the lungs of a patient whose symptoms were those of pulmonary tuberculosis. Warthin and Olney<sup>10</sup> collected a group of five such cases. This organism (*Streptothrix eppingeri*) has true branching threads occurring in large entangled masses, even grossly visible as minute grayish granules in a white, homogeneous, not bloody sputum. Some of the filaments are very long, thick, with short branches and without club-shaped ends. They are acid-fast, staining with a beaded appearance, but are slowly decolorized by 95 per cent. alcohol. They stain by Gram's method.

The *LEPTOTHRIX* group of normal mouth organisms flourishes in abundance in the lungs, especially in putrid gangrenous disease. Their probable effect is to aid in the decomposition of the sputum. Miller has separated from the old group of "*Leptothrix buccalis*," *Leptothrix innominata*, an organism unsegmented, straight, but sometimes wavy, from 0.5 to 0.8 micron broad, which occurs always in the tartar of

\* Mt. Sinai Hospital Reports, 1907; Centralbl. f. Bakt., Orig. vol. 41, p. 314.

<sup>9</sup> Flexner, Johns Hopkins Hosp. Bull., June, 1897.

<sup>10</sup> Am. Jour. Med. Sci., 1904, cxxviii.

teeth. This cannot be cultivated, and with iodine solution stains a pale yellow. *Bacillus buccalis maximus* is an organism occurring in single threads or in bunches of parallel threads, the single organisms from 30 to 150 microns long and 1 to 1.3 microns broad, joined into long threads. These cannot be cultivated and take a deep blue with iodine. *Leptothrix maximus buccalis*, a somewhat longer parasite than the last mentioned, but otherwise similar except it does not give the iodine reaction.

The MICROCOCCUS TETRAGENUS is a parasite occurring always in groups of four in a mucous capsule, each organism about 1 micron in diameter. It is a pyogenic parasite which occurs in the sputum in bronchitis, in tuberculous cavities, and hemorrhagic infarctions. It may aid the tubercle bacillus in its destructive processes. To recognize the pathogenic form it must be cultivated, for there is in the mouth of normal persons a harmless parasite of exactly the same appearance, which, however, cannot be cultivated.

SARCINÆ are rare in the sputum, and when they do occur are probably harmless saprophytes. They occur chiefly in gangrene, tuberculosis, bronchitis (see page 74), pneumonia, and in the sputum of old debilitated persons. They are the cause of the gray patches of stomato-pharyngomycosis sarcinica. Whether this parasite is the same as the sarcina ventriculi or not is a disputed point.

YEASTS occur but rarely are recognized. Fresh sputum must be examined. They are usually accidentally present, since, although universal, they flourish only in the fluids suitable for them, and in man these fluids are rare excepting the urine of diabetes and the gastric juice in certain cases of dilatation. They occur often enough, however, and should not be overlooked or unrecognized. They are oval or elliptical cells (see Fig. 64), rarely spherical, so refractive that they may resemble a fat droplet so markedly that chemical reactions are necessary to differentiate the two. Their size varies from 1 to 40 microns in diameter, although of each yeast there is a recognizable average size. Their appearance varies, in some cases being naked cells, in others with a membrane, or a membrane and vacuoles, according to the age of the cell. In some the nucleus is evident even in the fresh cell. The characteristic feature of a yeast is its reproduction by budding; that is, the projection from any part of the cell of a small bud which grows and then constricts off. Although for the most part extraneous, the presence in the sputum of certain pathogenic yeasts cannot be denied, and in doubtful lung cases they should be looked for. Busse<sup>12</sup> has discussed at length his case of "saccharomycosis hominis," an infection by a pathogenic yeast, "*Saccharomyces busse*," of the tibia, resulting in caseous cavities in both lungs in which the yeast

<sup>12</sup> Kolle and Wassermann, Handb. der path. Mikroorg., p. 669.

was present. The yeast cells were rather small, about 8 microns as an average size although they varied much, very refractive, resembling fat droplets except with a greenish shimmer. The younger cells were homogeneous, but later their membrane and granular protoplasm could be clearly seen. They are made clearer by the addition of sodium hydroxide. It is possible that did we search for such yeasts and not pass them by at once as simple saprophytes, more such infections could be found among our anomalous lung conditions.

MOULDS are commonly enough found, since the air is simply alive with their spores. For their recognition the examination of the fresh specimen is indispensable. Many of them are truly pathogenic, and it is remarkable that there are not more cases described as primary infections. Occurring chiefly in the ear of man, their next seat of predilection is the lung.

These moulds are found only in destructive processes of the lung. Whether these "broncho-pneumonomycoses" are primary or secondary has been a much disputed point with the weight of evidence at present in favor of the primary nature of certain of them. According to the former idea (Virchow) they were only secondary, or formed cavities in the areas of hemorrhagic infarctions. It is a peculiar thing that the cavities containing them are odorless, and there would seem to be an antagonism between moulds and the bacteria of decomposition so that a cavity filled with the former is protected against the latter, and *vice versa*. Granted a destructive process, these moulds would certainly aid and could crowd out the primary organism, hence when examined the case will appear to be one of primary mould infection. Recently, however, through the work of the French, also of Saxer and others, it seems probable that *Aspergillus fumigatus* can be the primary invader, causing by necrosis an odorless cavity. Among these moulds are:

(1) *Mucor*, of which there are one hundred and thirty varieties, six of which are known to be pathogenic. This is a very common air form. It is characterized by a much-branched unicellular mycelium, which later, however, may have septa; by the form of the sporangia which are at the end of erect hyphæ, and consist of a columella surrounded by the spores, the whole enclosed by a membrane. Fig. 9 represents *Mucor mucedo*, a very common harmless form. If such a mould be found in the sputum, it is well that the observer note carefully the shape of the columella, the size of the spores, and the nature of the membrane, although for certain recognition cultures are necessary. The varieties known to be pathogenic are *Mucor corymbifer*, which is a fine delicate small mould with spores 2 by 3 microns in size, the sporangia colorless, pear-shaped, and of a great variety of size from 10 to 70 microns; its membrane transparent.

The columella, evident only when the spores have dropped off, is top-shaped the large end distal, and colorless. This form has been found perhaps most often in man as the cause of kerato-, oto-, pharyngo-, and pneumonormycosis. *Mucor rhizopodiformis*, of which the sporangia-bearing hyphæ are single or branch as in a sheaf, short and of a brownish color. The sporangia are globular, when ripe of a black color, with an opaque membrane, soluble in water, and columella which is brownish, from 50 to 75 microns wide, constricted at the base, which is also truncated and with a wide flat apophysis, to the margin of which the membrane is attached. The spores are colorless, spherical, and from 5 to 6 microns in diameter. *Mucor racemosus*, the spores of which are from 5 to 8 microns long and 4 to 5 microns wide and round; the columella elliptical in shape. *Mucor pusillus*, the sporangia of which are black with a thorny membrane, and from 60 to 80 microns wide; the columella egg-shaped or spherical, light brown, from 50 to 60 microns wide; and the spores very small, round, colorless, from 3 to 3.5 microns in diameter. *Mucor septatus* has a pale, grayish-brown, spherical sporangium, small colorless columellæ which after the loss of the spores may grow still further. The hyphæ have septa, hence the name. The spores are about 2.5 microns in diameter. *Mucor ramosus*, the sporangia of which are 70 microns in diameter, black in color, with a transparent membrane; the columella round, the spores colorless, opaque, from 3 to 4 microns wide and 5 to 6 microns long.

These forms are known to be pathogenic; almost all of them have been demonstrated in the ear. It is interesting that in all literature only four cases are cited in which they have been demonstrated in the lung, and so far as we know in none of these cases were they found in the sputum before death.

*Aspergillus fumigatus* (see Fig. 10). This is by far the most important pathogenic mould. Its mycelium is a thick mesh of threads from 3 to 6 microns wide, the finest without but the oldest with septa. The conidia-bearing hyphæ are short, club-shaped, and from 8 to 10 microns in diameter at the larger (distal) end. The sterigmata are unbranched, from 6 to 15 microns long, and are packed together from a central point, thus giving a fan-like appearance. The conidia, a chain of which is at the end of each of the sterigmata, are round, colorless, and from 2.5 to 3 microns in diameter. All parts of this mould have a brownish to a dark grayish-green color. The size of the spore is important, since those of *Aspergillus glaucus* are from 7 to 8 microns in diameter. The spores occur everywhere, as can be demonstrated by exposing a moist piece of bread to the air for only a few minutes and then placing it in the thermostat. *Aspergillus flavus* (see Fig. 11), has conidia-bearing hyphæ which are from



FIG. 9.—*Mucor mucedo*.  $\times 600$ .



FIG. 10.—*Aspergillus fumigatus*.  $\times 300$ .



FIG. 11.—*Aspergillus flavus*.  $\times 300$ .





7 to 10 microns thick, with the head of a yellowish or green color, according to whether it is dry or wet, and brown when old. The conidia themselves are round, of a sulphur-yellow color and from 5 to 7 microns in diameter. *Aspergillus niger* is of a chocolate brown color, and the conidia are from 3.5 to 5 microns in diameter. *Aspergillus subfuscus* is of an olive-green to a black color, resembles much the *fumigatus*, but is more pathogenic. Of these forms the *Aspergillus fumigatus* is the only one that has been shown to bear a direct relation to that pathological process known as "pneumomycosis aspergillina." Sticker has collected from the literature twenty cases in which no other disease of the lung was present. Of these, in sixteen was found *Aspergillus fumigatus*, in four cases the mould was doubtful. One of these, reported by Osler, was a woman who for twelve years had expectorated masses of mycelium the size of a bean, grayish and of a downy consistency; in five cases the mould could not be classified. An interesting case of primary chronic "membranous" bronchitis due to the *Aspergillus fumigatus* was reported by Devillers and Renon.<sup>13</sup> The patient was a grain-sorter. Fragments of membrane composed of the mycelium of this mould (recognized from cultures) were expectorated monthly. They were from 1 to 6 cm. long, and, having no branches, probably arose in the larger bronchi. In nineteen cases with the *Aspergillus fumigatus* the infection was mixed. Sticker<sup>14</sup> has divided the cases into the "sporadic," which are of old feeble subjects or persons suffering from a lung disease, and the "endemic," in which case the disease is due to the occupation of the patient. The two best illustrations of this latter have been described by the French writers. The first consists of a pseudo-tuberculosis present in pigeon-feeders, who are much exposed to the moulds of grain, and hair-combers who work in an atmosphere so laden with infected dust that the cat is the only animal that can live in their neighborhood. No autopsies have been made on such cases. Clinically, the course is often similar to that of a chronic pulmonary tuberculosis. At the onset there is hemorrhage in some cases, either slight or profuse, and which is generally repeated at intervals. The cough is dry at first, then accompanied by a frothy sputum which quickly becomes greenish in color and purulent. This may continue for months or even years. Blood flecks are often present. Toward the end the hemorrhage may recur or not, the expectoration is of a greenish color, purulent in nature, and nummular in character.

Another form is a chronic bronchitis resulting in final cirrhosis

<sup>13</sup> La Presse Med., 1890, ii, p. 325.

<sup>14</sup> Schimmelpilzkrankh. der Lungen, Nothnagel's System, 1900, xiv.

of the lung. In this case the sputum is abundant, foamy, and watery. In Wheaton's case<sup>15</sup> the condition simulated actinomycosis with anatomically the presence of a few tubercles and a large cavity. In some cases there have been casts of the bronchi formed of mycelium and conidia expectorated.

For diagnosis these moulds must be demonstrated in the sputum, and in any case of suspected tuberculosis without the tubercle bacillus they should be searched for. There may be present either the mycelium, the conidia hyphæ, or the spores. As a rule, they are overlooked or passed by as extraneous. The odorless character of the sputum is an interesting fact, even in cases of marked gangrene of the lung with the expectoration of large masses of lung-tissue. In some cases the absence of the mycelial threads may be explained by their destruction, which certainly soon occurs.

The *Penicillium glaucum* (see Fig. 12), which is the most common of our media contaminations, has segmented conidia-bearing hyphæ which divide brush-like at the end, the branches being tipped by sterigmata which are flask-shaped, bearing conidia from 2 to 3 microns in diameter. The refractile conidia of this mould are so common that it is strange that the students do not recognize them oftener, particularly as some show the characteristic sprouts. The mould is non-pathogenic. The *Penicillium nummula* is certainly pathogenic for animals, and has been found in the ear of man.

Although the general class, mucor, aspergillus, or penicillium may be recognized if the sporangium or the conidia head is found, yet a closer differential diagnosis can only be made on cultures. This may be done by spreading the sputum over a piece of bread as media, or using Sabourand's media (maltose, 3.7; pepton, 0.75; water, 100).

The moulds may be stained in the fresh specimen by a saturated watery solution of saffranin or, better still, of thionin.

**BLASTOMYCOSIS.\***—Blastomycetes are budding protophytes belonging to the same group as yeasts (Fig. 20, page 92). These organisms, round or oval in shape, and from 8 to 12 microns in diameter, consist of finely granular, often vacuolated, protoplasm, and a doubly contoured capsule separated from the protoplasm by a clear zone, often wider on one side than on the other. Reproduction in the living tissue is by budding only; in cultures, by mycelial formation.

These parasites grow well on glycerine, glucose-agar, blood serum, bouillon, and other ordinary media. The growth is microscopically visible in from 2 to 14 days. The growth at room temperature is

<sup>15</sup> Trans. Path. Soc., Lond., vol. xlv, p. 34.

\* See Montgomery and Ormsby, Arch. Int. Med., Aug., '08, vol. ii, No. 1, p. 1; also Fontaine, Haase, and Mitchell, Arch. Int. Med., Aug., '09, vol. iv, No. 2, p. 101.

dry, with an abundant development of aerial hyphæ; that in the incubator is pasty and moist, with less aerial growth. In the cases reported thus far the organism has been first discovered in cultures made from the pus of subcutaneous abscesses and from blood cultures, later in other secretions.

The organism is pathogenic to rabbits, producing systemic infection if injected intraperitoneally or intravenously. Subcutaneous inoculations are usually unsuccessful.

Thus far but 23 cases of blastomycosis have been reported, the majority among foreign-born patients in Chicago. The symptoms suggested tuberculosis, and the correct diagnosis was seldom made before the subcutaneous abscesses had appeared. In practically every case of this disease the lung is involved sooner or later and the



FIG. 12.—*Penicillium glaucum*.  $\times 300$ .

evidence would indicate that in at least 65 per cent. of all cases the primary infection is in the lung.

The organisms may be found in the sputum, blood, urine and in the pus of the subcutaneous abscesses. In the sputum they are found in enormous numbers, and probably early in the history of the case. Thus far no first diagnosis of a case has been made from sputum examination, which shows how seldom we examine fresh sputum, or, perhaps, how little we see when we do examine it. The characteristic sputum is abundant and very blood-stained. The patients, however, do not expectorate such sputum all the time, but for long periods only a mucopurulent sputum.

**Coccidiosis.**—Thus far but few cases of infection with *Oidium coccidioides* have been reported.\*

\* Hektoen, Jour. A.M.A., Sept. 28, 1907, vol. 49, p. 1071. In this paper seventeen cases are reviewed.

*Oidium coccidioides* is one of the budding fungi belonging to the same group as the yeasts and the blastomycetes. It differs from the latter in that, while the blastomycetes multiply in the living tissues only by budding, this organism multiplies by endogenous spore formation.

Most patients with this infection belong clinically to the pseudo-tuberculosis group, since early in their infection all have had primary lung involvement, bronchopneumonia, or pulmonary abscess with purulent or blood-streaked sputum, which in several of the cases contained the organism.

*OIDIUM ALBICANS* is the very common parasite of thrush, which can develop in the lungs as well as at its common seat, the mouth and pharynx. It occurs chiefly in the mouth of children during their first week, especially in the weak babies; more rarely in older children or adults weakened by old age or disease, especially diabetes or typhoid fever. In these cases we have secondary growths in the throat, nose, œsophagus, bronchi, and lungs. The most common form is the large-spored variety. It may occur in the sputum in two forms,—the first, the yeast-like cells, from 5 to 6 microns long and 4 microns wide, and oval, which in shape cannot be told from any other yeast; and the threads of all sizes and lengths with a double contour containing droplets, granules, vacuoles, but especially conidia-like bodies which are true endogenous spores.

*ACTINOMYCOSIS INFECTION* of the human lung is rare. It sets up a chronic process, but one which progresses unrelentingly till death. In some cases there is a slight catarrhal bronchitis for a long time, but the most common form is, from the onset on, a bronchopneumonia. The consolidated areas break down forming cavities which contain fluid, pus, fatty detritus, fat globules, degenerated red blood-cells, and the sulphur granules. Clinically, the picture is of tuberculosis, and yet if the sputum be watched carefully an early diagnosis can be made. Other cases are of chronic bronchitis, while still other cases present the picture of a miliary tuberculosis. The abscess cavities may be very large. The sulphur granules are the characteristic find. They are small granules, in size varying from microscopic to 2 mm. in diameter, of a yellowish, grayish, greenish, or brownish color, round, sometimes abundant in number, in other cases, few. Microscopically they are a net-work of fine twisted threads, straight or wavy; at the ends of many at the periphery are the characteristic club-shaped swellings which when present in large number form a ring around the granule to which they give a radiating or star-like appearance. In general it may be said that in any case of atypical lung disease always think of this (Osler). The expectoration is usually mucopurulent, sometimes fetid. It may be simple mucus, very scanty, or may be purulent and hemor-

rhagic. It is said that the sputum is sometimes as rusty as in pneumonia. It is also possible that the patient may say that he has expectorated at one time a large amount of offensive yellow material. Tubercle bacilli will not be found, neither will elastic tissue.

**Animal Parasites.**—INFUSORIA are rare and unimportant. Artault described *Amœba pulmonalis* as "a small amœboid cell which when dead and stained looks exactly like a leucocyte, but while motile differs from it in its refractivity and staining qualities." *Amœba coli* is frequently found in the sputum of cases of liver abscesses which have perforated into the lung and in cases of abscess of the jaw communicating with the mouth (Flexner). It is important that the student should bear in mind that the same rule obtains here as in the examination of the stool, and that nothing should be called an amœba unless its amœboid motion has been clearly seen.

Of the FLAGELLATA, *Trichomonas pulmonalis* is the name given to a form which has been found several times in the sputum. A. Schmidt found them only in the Dittrich's plugs, while Artault found them in the contents of a large tuberculous cavity. These may be the forms which others have found in lung gangrene and putrid bronchitis. In a recent case of large abscess of the lung following pneumonia with operation six weeks after the onset of the pneumonia, the sputum contained large numbers of these flagellates. It is probably the same as *Trichomonas vaginalis*. *Cercomonas* have been found in the sputum and in the Dittrich's plugs of lung gangrene.

**ECHINOCOCCUS DISEASE.**—Next to the liver the lung is the most common seat of this infection (in 4.5 per cent. of cases) (see Fig. 55). If the cyst bursts we may find in the sputum the daughter cysts, scolices, hooklets, or fragments of membrane, any one of which is characteristic. They may also be derived from a liver cyst which has perforated through the lung. The cyst wall consists of two layers, —an external laminated cuticular capsule, and an internal granular parenchymatous endocyst. Fragments of this laminated capsule are characteristic of the disease. The cyst content is a clear, limpid fluid, from 1005 to 1015 specific gravity, which contains no coagulable albumin, is neutral or slightly acid, and contains considerable sodium chloride. Inosite, leucin, tyrosin, and succinic acid may be found, also hæmatoidin. From the endocyst develop buds which grow into smaller daughter cysts, and which break loose and lie free in the parent cyst. Inside these daughter cysts may develop granddaughter cysts. From the inner wall of any of these cysts may develop brood-capsules, cysts from the inner or outer wall of which the scolices develop. A scolex is the head of a *Tinea echinococcus*, and presents a rostrum with four suckers and a circle of hooklets. If found while alive, the head actively protrudes and retracts this rostrum. In many cases the cyst

wall degenerates, becomes inspissated and filled with a cheesy material which contains masses of free hooklets and dead scolices. The latter have a coating of calcium carbonate which effervesces actively on the addition of hydrochloric acid. The presence of these cysts may lead to gangrene of the lung and the formation of cavities connected with the bronchi. Hemorrhages are the rule, usually slight or mere streaking, but sometimes profuse. As a rule, such cases are diagnosed as phthisis or gangrene, unless one of these characteristic bodies is found in the sputum. The cough may at first be dry and hacking, and then as the cyst increases in size there may be some mucoid expectoration. If the cyst ruptures its cavity becomes infected and the sputum then is fetid pus, in some cases of a chocolate color which resembles the pus said to be characteristic of hepatic abscess. In other cases the suppuration occurs before the rupture and the contents undergo fetid decomposition; then follow the symptoms of rupture of an abscess. If daughter cysts and pieces of membrane are found in the sputum, it means that the bronchus communicating with the cyst is a large one. Pieces of the membrane may be expectorated for months. The rupture is often accompanied by a copious hemorrhage which later may recur.

**PARAGONIMUS WESTERMANII.**—This parasite, the "lung fluke," is the cause of the parasitical hæmoptysis of man which occurs so com-

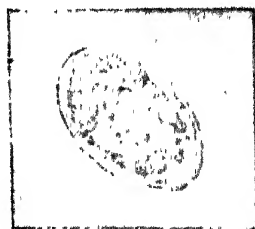


FIG. 13.—Egg of *Paragonimus westermanii* from the sputum of Dr. Mackenzie's case (through the kindness of Dr. Stiles). · 400.

monly in Japan, parts of China, and Korea. In some mountain towns a majority of the people are said to be infected; in Okayama, 0.4 per cent. of all hospital cases admitted; in Kumamoto, 5.0 per cent. of pulmonary cases.<sup>16</sup> One case in this country which came under the care of Dr. Mackenzie, of Portland, Oregon, has been reported by Stiles,—that of a Japanese who recently immigrated. There was found in domestic animals in this country, previously, a parasite which seems to be identical, and it is perhaps only a matter of time before more and endemic cases will be found among our cases of "tuberculosis." The duration of the disease is long, from ten to twenty years from the appearance of the first symptom. The diagnosis rests with

<sup>16</sup> Inouye, Zeits. f. klin. Med., 1903, vol. 1, p. 120.

the discovery of eggs in the fresh sputum. The sputum is generally small in amount, very viscid, and consists of small pellets of blood mixed with mucus. In other cases it is as rusty as that of pneumonia. When no blood is present the sputum may have any shade of yellow and brown, but especially a dark, dirty red or brown, a color due to the eggs themselves. Colored spirals resembling grossly the Curschmann spirals are quite characteristic. The eggs are the only characteristic symptom, and may be expectorated in large numbers. The amount of blood is usually small, at the onset only a few drops, but it may be large, from 300 to 800 cc. in a few hours, especially if the patient leads a laborious life. There may be rather large hemorrhages which recur with great frequency, or the disease may take a very slow course. The hemorrhage is always arterial. Its cause is not clear, and it seems more accidental than otherwise, since the ova are not in the pellets of the blood, but in the rusty portions. The eggs have a thick, smooth shell of a dirty reddish-brown color with a characteristic lid at one end. In some eggs one does not see the lid, in others it is not exactly on the end, and in others it is partly shelled off. They are 68 to 96 microns long, 48 to 60 microns wide. Charcot-Leyden crystals are common in the sputum, "sufficient proof that such crystals do not explain asthmatic paroxysms, since these cases never have asthma." <sup>17</sup>

Now that a case of this disease has been found in America it is very desirable that all be on the watch for more.

**Chemical Examination of the Sputum.**—The chemical examination of the sputum is seldom of much importance, and those tests which have been proposed have as their object to demonstrate the relative amounts of mucin and albumin. The Zenoni modification of Schmidt's method (Schmidt used the Grübler-Biondi stain) is perhaps the most valuable of the muco-chemical tests. Zenoni spreads a small particle of the sputum on the cover-glass, treats it with alcohol for at least a quarter of an hour, and then stains with a half-saturated water solution of safranin. The specimen is examined against a white background. The mucus stains yellow, and the albumin red. This method has the advantage that when many pus-cells are present, the color of the ground substance can be determined microscopically. In pneumonia there is much more albumin than in other conditions.

The chemical test for soluble albumin is simple. The sputum is mixed with 3 per cent. acetic acid, shaken well, allowed to stand twelve hours, and filtered. The filtrate is tested with potassium ferrocyanide, or neutralized, sodium chloride added, and the heat-acid test used. Quantitatively it may be estimated by the Esbach tube. In the filtrate

<sup>17</sup> See Stiles and Hassall, Sixteenth Report of the Bureau of Animal Industry, 1899.



of the heat test the albumoses may be precipitated by zinc sulphate, or determined by the nitrogen present. Deutero-albumoses alone have been found; no peptone (Wanner). Wanner found by far the most soluble albumin in the sputum of pneumonia,—0.3 to 3.0 per cent.; least in that of bronchitis (a mere trace); in that of practically normal persons the merest trace, if any at all. Its presence means inflammation, not hypersecretion alone. Anything more than a faint opalescence is pathological.

Mucin he determined from the glucosamin formed by adding to a weighed amount of sputum two volumes of alcohol, shaking, filtering through a hardened filter paper, and washing with alcohol. The precipitate is then boiled with 10 per cent. HCl for three hours in a flask with return-cooler. The flask is then quickly cooled, made alkaline with NaOH, then acid with acetic acid, then precipitated with phosphotungstic, to remove the biuret-giving bodies, and the reducing substance determined with Fehling's solution (glucosamin having the same reducing power as glucose). Pure mucin contains 33.6 per cent. glucosamin.

Much mucin (1 to 3.3 per cent.) was found in chronic bronchitis, a moderate amount in pneumonia (0.66 to 1.03 per cent.) and phthisis (0.74 to 0.79 per cent.), none in bronchiectasis. Sputum is digested rapidly by autolysis.

As to the value of these chemical tests, which are easy and rapid; Wanner considers that a definite trace of albumin in a case of incipient tuberculosis or chronic bronchitis will mean the former; much albumin indicates pneumonia or pulmonary cedema, and in a case of either pneumonia or infarction of the lung, the former.

Peptone in the sputum has been claimed by some, denied by others, and a trypsin-like ferment is assumed in gangrene of the lung to explain the partial disappearance, perhaps complete in some cases, of elastic tissue.<sup>18</sup>

**Pulmonary Tuberculosis.**—"Pulmonary tuberculosis has no characteristic form of sputum" (Brown).

Cases of the FIBROID FORM may have no or little expectoration, and this free of tubercle bacilli for a long time. But, as a rule, there is a purulent sputum.

In ACUTE MILIARY TUBERCULOSIS the sputum, if any at all, is that of bronchitis, hence mucopurulent or blood-streaked. No tubercle bacilli need be present. In the ACUTE PNEUMONIC TUBERCULOSIS with extensive caseous consolidation there may for one or two months be no sputum whatever, but, as a rule, it is that of a typical acute lobar pneumonia, rusty until the crisis should come, and then when one expects

<sup>18</sup> See Wanner, *Deutsch. Arch. f. klin. Med.*, 1902, lxxv. 347; Fr. Müller, *Ztschr. f. Biol.*, Bd. 52. In this is the best discussion of the mucin and allied bodies of the sputum.



it to change to a mucopurulent a green color may be the first indication that there has been a mistake in diagnosis. In a case, therefore, of pneumonia with delayed resolution, and especially if the sputum be green, search should always be made for tubercle bacilli and elastic tissue, for sooner or later they will be present, and sometimes very early.

Among our cases of acute tuberculous lobar pneumonia (fifteen in number), in four the sputum was typically rusty. In the majority it was a mixture of a rusty with a bronchitic; in two there was almost no sputum at all. The green color and tenacious quality were marked in a few; in two cases the sputum was of a white, sticky, mucopurulent nature from the very first; one marked feature in nearly all cases was the constant blood-streaking, and in two, brisk hemorrhages. In the typically rusty sputum very few pus-cells were present, but many alveolar epithelial cells and red corpuscles. Later, that is after the first week, the sputum is rather mucopurulent, and yet in many cases it continues blood-streaked. The greenish color was marked in several. Later it became nummular, and in two cases positively foul.

In cases with a slight bronchitic sputum which develop tuberculous pneumonia the sputum at once changes its nature, becoming tenacious, slightly less in amount, and blood-streaked. If a true acute lobar pneumonia occurs in the course of a chronic tuberculosis, the sputum is that of lobar pneumonia with an admixture of the bronchitic.

Among our cases the following points were particularly interesting: In one the mixture of the rusty and bronchitic sputum expressed itself by the two layers in the cup, the upper mucopurulent and blood-tinged, the lower exceedingly tenacious and stringy; later on it became greenish, purulent, and very tenacious, then of a tenacious greenish-gray color, which continued until death a short time later. Another case is interesting, since the elastic tissue was found before the tubercle bacilli, although repeated examinations for the latter were made. In one case diagnosed at first as acute lobar pneumonia, on the third day the sputum was markedly blood-tinged, tubercle bacilli were present, and two bronchial casts about 1 mm. in diameter at their larger end were found. In a case with sudden onset two days before admission and without previous history the sputum was white, sticky, and mucopurulent; this continued with a greenish tinge for nine days, but on the nineteenth day there was a sudden marked change, the sputum now abundant forming easily two layers, the upper sanguineous, the lower mucopurulent, blood-tinged for the first time, and on that day, the nineteenth, the tubercle bacilli were first found, although they had been repeatedly searched for.

In the ACUTE TUBERCULOUS BRONCHOPNEUMONIA a hemorrhage is sometimes the first symptom. The sputum may early show elastic tissue and tubercle bacilli.

In the CHRONIC ULCERATIVE TUBERCULOSIS, the sputum may assume almost any color or any form assumed in any other disease. Biermer divided it into four forms; the mucoid, mucopurulent, blood-stained, and pure blood. It may vary in amount from nothing to one litre in twenty-four hours; in consistency from that of extreme tenacity to very watery. In some cases, especially those of the early

apex tuberculosis in which the physical signs are marked and a cough present, there is no sputum. For weeks there is usually a slight morning sputum, but it may be necessary to urge the patient to expectorate this; in other cases the onset is with a slight hemorrhage; in other, it is at first a pure mucus and hence glairy, containing much myelin, giving it the sago appearance. There is nothing distinctive in the gross appearance of this sputum. It may last for months, but sooner or later there will appear little caseous lumps, one of the first suggestive signs of tuberculosis. Later the sputum is more profuse and mucopurulent, that of chronic bronchitis. As ulceration proceeds it becomes more profuse, yellow or greenish in color, and after that of any grade or amount, and from mucopurulent to purulent, or even pure pus. Microscopically, it contains epithelial cells of all kinds, pus-cells, blood, and in some cases, if the sputum be hardened *en masse* and sections cut, giant-cells may be found.

The *variations* in the sputum in chronic ulcerative tuberculosis are many. "A sudden disappearance of the sputum when before it had been abundant, especially in the morning, should always put us on our guard. Miliary tuberculosis is occasionally ushered in in this manner" (Brown). In cases of sudden heart failure there may be a cessation of sputum for one or two days without apparent injury. An abundant mucoid more or less frothy sputum marks the onset of miliary tuberculosis in a chronic or a less acute form (Brown).

One of the most important points in the sputum examination is the recognition of the small caseous particles, "rice bodies" (*corpora oryzoidea*). The sputum should be spread out on a dark plate, or squeezed between the surfaces of two plates of glass, and then the whole surface scrutinized with a small hand-lens. Many prefer wide Petri's dishes, which are more easily handled and sterilized. This is the surest way to find these particles, in which one has the best chance of finding tubercle bacilli and elastic tissue. These small caseous particles are from about 0.5 to 1 mm. in diameter, of a white opaque color, more or less rounded in shape, of a bad odor, and when picked up with a needle and spread on a slide are found to be more brittle and crumbly in character than particles of food.

ELASTIC TISSUE (see page 29).—The search for this should never be at random. It should always be methodical and intelligent, for one can search long and find none, whereas, did he know what particles to choose, he might find plenty in the first specimen. Its presence means destruction of the lung. As a rule, in tuberculosis the disintegration is molecular and the elastic tissue in very fine particles, grayish threads, even in single fibres. These fibres may present the arrangement of the alveoli, or come from the bronchi or from the blood-vessels. In certain cases it will be found early before there is any suspicion of



FIG. 14.—Tubercle bacilli, stained with carbol fuchsin and decolorized with nitric acid.  $\times 900$ .



disintegration; in other cases death may ensue before any is found, as, for instance, in caseous pneumonia.

The constant presence of elastic tissue in the sputum means that there is an advancing destructive process in the lung. When healing begins, the elastic tissue diminishes and finally disappears.

TUBERCLE BACILLI (see Fig. 14).—The search for these, the most important proof of tuberculosis, should be made with especial care. The caseous particles already mentioned should be selected if present, and if none are found, smears are made from the small bloody or purulent masses. The bacilli may be present in the bloody masses of the initial hæmoptysis. One must not give up in case no promising particles are found, for bacilli may occur in goodly numbers in the watery mucoid sputum as well as in the mucopurulent.

In case but very few bacilli are present we may improve our chances of finding them by rendering the sputum homogeneous and fluid, and then precipitating the bacilli to the bottom of the tube, or salting them to the top. The best quick method of obtaining a sputum sufficiently homogeneous and fluid is Löffler's\* modification of Uhlenhuth's method. A measured quantity of sputum (5, 10, or 20 c.c.) is mixed in a Jena flask with an equal quantity of 50 per cent. antiformin (which contains NaClO and NaOH) and boiled not over 15 minutes. The solution will foam considerably and become somewhat brownish. For each 10 c.c. of the fluid are now added 1.5 c.c. of a mixture of chloroform (1 part) and alcohol (9 parts). The whole is then shaken vigorously until a fine emulsion is produced, when some is poured into centrifuge tubes and precipitated for 15 minutes. The heavier constituents, including some of the bacilli, collect in a film just above the chloroform. The supernatant fluid is poured off, the film is removed in toto and put on a glass slide, and the excess of fluid is absorbed by filter paper. A drop of egg albumin mixed with carbolic acid (enough to give a 0.5 per cent. solution) is then added, and the sediment is spread by a second slide into a thin smear, allowed to dry in the air, fixed in the flame, and stained.

The sputum may easily be liquefied by boiling a measured portion with an equal volume of 5 per cent. KOH, but this injures the staining properties of the organisms. Or the sputum may be digested. From 5 to 10 c.c. of sputum are mixed with ten volumes of 0.2 per cent. soda, 0.5 gm. of pancreatin is then added and the mixture left in the thermostat for from six to twenty-four hours. A little phenolphthalein is added that one may be sure the reaction remains alkaline. Of these two methods the first is the least useful, since it changes the staining properties of the bacilli.

\* Dent. Med. Wchs. Oct. 27, 1910. Vol. 36, No. 43, p. 1987.

When attempting by means of a centrifuge to obtain from any fluid a sediment containing all the organisms in that fluid, one should remember that the reason why we ever succeed at all is that so many of the bacilli are enclosed in small masses of sediment which can be thrown to the point of the tube. The specific gravity of tubercle bacilli lies roughly between 1010 and 1080, and if, as so often happens (for the specific gravity of sputum varies from 0.929 to 1.2242), the specific gravity of the fluid is greater than that of the bacilli, the centrifuge will defeat its own object, and more organisms will be formed at the top than at the bottom of the tube. In fact, eight times as many bacilli have been found in the supernatant fluid as in the sediment. By diluting the fluid with an equal volume of alcohol one usually gets a mixture of so light density that even the free bacilli will sink to the bottom; but some prefer to add an equal volume of a 25 per cent. NaCl solution and allow the mixture to stand for 24 hours. In this heavy liquid the bacilli will rise to the top.

The old method was to make the smears on cover-glasses, but now most prefer slides, because on them one can make a much larger smear, and these specimens need no cover-glasses. It is always wise to fix the organisms to the glass by a little carbolyzed egg albumin (see above). The sediment may be spread by a second slide, a needle or a hatpin. While making the smear, one should hold the slide at such distance above a flame that it is slightly warmed, for the mucus is then more easily spread.

**STAINING.**—As a routine method the following is recommended. We use here as few words as possible, but the reader is urged to study carefully the paragraphs which follow. The specimen, spread, dried, and fixed on a slide, is covered with the Ziehl-Neelsen carbolfuchsin solution and heated over a small flame. It is necessary to make frequent additions of stain with a dropper; otherwise the specimen will dry, and the glass crack. The staining solution covering the smear should boil about a minute. The stain is then poured off, and the smear is washed in water and dried with a blotter. The smear is then decolorized with acid alcohol until it is practically colorless. Next, it is washed in water, blotted, and kept covered with Löffler's methylene blue about five seconds. After the excess of this stain is washed off, the specimen is dried with a blotter and mounted. Or, one can put a drop of immersion oil directly on the dried smear and use no cover. On examination the tubercle bacilli will be found stained red, and all other organisms blue.

The possibility of an almost specific stain for the tubercle bacillus depends on the fact that this organism is both acid-fast and resists decolorization by alcohol.

We now will discuss the method more at length. The entire

specimen is first deeply stained with a penetrating dye which will stain all bacteria. It is then decolorized with an agent which will remove the stain from practically all organisms except *Bacillus tuberculosis*. If the smear is then counterstained, the tubercle bacillus alone will retain the red color, while practically all the others will become blue. It is, however, difficult to get the tubercle bacillus to take any stain at all. The Ziehl-Neelsen carbolfuchsin mixture is the one in common use. (Fuchsin, 1 gm.; absolute alcohol, 10 c.c.; 5 per cent. carbolic acid, 100 c.c.) The specimen can be stained by heat or in the cold. If the latter is used, the specimen is left submerged in this stain for twenty-four hours. If heat is preferred the slide or cover-glass, whichever is used, may be submerged in a flat dish containing the stain and heated over a free flame; or the cover, held in a suitable forceps, and the slide, if that is used, held in a holder, or in the fingers, is covered with the stain and heated over the free flame. It is necessary to keep renewing the evaporating carbolfuchsin.

The staining fluid must actually boil, or the tubercle bacilli will not take the stain. For it merely to steam is not sufficient. It is usually enough for it to boil from a quarter to half a minute; but, to be on the safe side, many prefer to let it boil from one to four minutes. With the best of technic probably but a fraction of the tubercle bacilli will take any stain; so that one must be careful that the initial staining be as complete as possible. Since high heat certainly injures the specimen, if very careful work is desirable, the cold, slow method is to be preferred.

There are several ways of decolorizing the specimen. The best reagent is acid alcohol [2 per cent. HCl (some say 3 per cent.) in 80 per cent. alcohol]. The slide may be dipped into a vessel of this fluid or the acid alcohol repeatedly poured on and drained off. The decolorizing process should be repeated until the specimen is to the eye practically colorless. It is hastened by warming the slide covered with the fluid. Many urge the watching of the process under the low power of the microscope, and this does enable one to determine very accurately when the specimen is sufficiently decolorized. Some advise that the specimen be decolorized with 25 per cent. nitric acid, and in the very popular Gabbett's method 25 per cent. sulphuric acid is used. The latter "burns" the specimen more, and makes the bacilli look thicker, and their beading, etc., less distinct than does nitric acid. The disadvantage of these two acids is that there are many acid-fast bacilli, several of which might be encountered in the sputum. These any method involving the use of acid as decolorizing agent would not differentiate from the tubercle bacillus. On the other hand, there are few acid-fast and alcohol-fast bacilli. For this

reason acid alcohol is by far the best decolorizing agent. Thin smears will be decolorized more rapidly than will thick, and the small masses which occur in smears will not easily be decolorized and may be disregarded.

After it is decolorized, the specimen is washed in water. If then any red tint returns, the acid is again applied. As counterstain Löffler's methylene blue (see page 290) is generally used. The specimen, after it is decolorized, washed in water, and dried, is kept covered with this counterstain about five seconds. Löffler himself uses a 0.1 per cent. solution of malakit green (Malachitgrün crystallen chlorzinkdoppelsalz, Hoechst).

In one of the commonest and easiest methods the decolorizing and counterstaining are combined. After the excess of carbol-fuchsin is washed off, the specimen is dried and kept covered with Gabbett's methylene blue (1 to 2 gms. of methylene blue, *i.e.*, enough to saturate, in 100 c.c. of 25 per cent. sulphuric acid) from 1 to 5 minutes. The Gabbett's solution is then washed off in water, and the specimen held up to the light. If any pink tint remains, the smear is again covered with Gabbett's solution and is again washed. The process is repeated until only the thick clumps retain any red tint. This method is fairly satisfactory in sputum examination, since it is relatively unusual for any other acid-fast bacilli to be found in the sputum; but in a doubtful case it cannot be trusted. If, however, one is merely following the sputum of a case of tuberculosis concerning the diagnosis of which there is no doubt, to determine from day to day any increase or decrease in the number of bacilli present, this is the easiest and therefore the best method.

Pappenheim<sup>19</sup> has recommended a stain supposed to be very superior for its differentiating qualities. This seems unnecessary, however, to those who have most experience in the practical side of the work.

His method is as follows: The specimen is covered with carbol-fuchsin and for a short time this is raised to the boiling-point. The excess of carbol-fuchsin is then poured off. The specimen is not washed, but is covered at once with a decolorizing and counterstaining solution which is prepared as follows: One part of corallin is dissolved in 100 parts of absolute alcohol. This solution is saturated with an excess of methylene blue, and 20 parts of glycerine are added. The solution is poured on and slowly poured off the specimen from three to five times. The smear is then quickly washed in water, dried, and mounted.

The tubercle bacilli are stained red by this method, and the smegma and other organisms, blue.

<sup>19</sup> Berl. klin. Wochenschr., 1898, No. 47.



By no means are all tubercle bacilli acid-fast. This fact probably explains our failure to find them in the sputum of certain patients who, without much doubt, have very active pulmonary tuberculosis. But since these tubercle bacilli which are not acid-fast are Gram-positive, Much recommends that we control our negative finds by staining these sputa by Gram's method.

*Morphology.*—The majority of tubercle bacilli found in the sputum are from 1.5 to 3.5 microns long and about 0.2 micron wide. Some, however, are even 11 microns long, and others which are longer still and branch resemble spirochæte. Many, even of the shortest, are somewhat bent, while the longer are usually very much curved. Chains of these bacilli are sometimes seen. *Bacillus tuberculosis* is not a spore-bearing organism. Contrary to the opinion held by many, this organism is as susceptible to heat as are *B. typhosus*, *B. coli communis*, and other bacteria which do not produce spores\* (*i.e.*, all are killed at 60° C. in 20 minutes). Tubercle bacilli will retain their virulence in dried sputum from three to ten months.

In stained specimens the bacilli are found scattered or in clumps, and in the clumps they may lie parallel or crossed.

Beaded forms (from one to eight beads in each rod) resembling streptococci are common. These are considered degenerated forms. They are found in all classes of cases. Some believe that the young bacilli stain less intensely than do the older forms, but this is now disputed. While various determinations show that other bacteria contain only from 1.7 to 10 per cent. of fatty matter, tubercle bacilli contain from 10 to 37 per cent. This probably has some influence on their staining characteristics.

The method of demonstrating tubercle bacilli in the sputum by animal inoculation has never been used as much as it should have been because of the fear that *Diplococcus lanceolatus* and other organisms common in the sputum would kill the guinea-pigs. Rosenau, however, informs me that he often uses this method and with good success.

The group of acid-fast organisms has recently, and with good reason, received careful study. Supposed at first to be the characteristic of but few bacteria, resistance to decolorization by acid is now known to belong to a large group, chiefly of non-parasitic organisms. Among these are (Borrel<sup>20</sup>) *Bacillus tuberculosis*, *Bacillus lepræ*, the smegma group, the "milk and butter" bacilli (Robinowitch), the timothy hay bacillus and the grass bacillus of Moeller, bacilli found in manure (Moeller), and a large group found in sewer water, in soil, etc. The majority of these organisms are

\* Rosenau, Bull. of Hygienic Lab., Sept., 1909, No. 57.

<sup>20</sup> Bull. Inst. Pasteur, May 30, 1904.

of much greater interest to the hygienist than to the clinical microscopist, altogether the interest recently aroused by the report that it is easy to find *Bacillus tuberculosis* in the circulating blood of consumptives (Rosenberger) demonstrated to the microscopist the necessity of examining his reagents, even the distilled water, for acid-fast bacilli.\* Several of these acid-fast organisms have considerable clinical importance, as they are often mistaken for the tubercle bacillus. Chief of these, since they so often lead to mistakes in diagnosis, are the smegma organisms which are found in those parts of the body where the secretions of the skin are allowed to collect, as around the genitals, in the axilla, in cerumen, etc. The smegma is particularly rich in these organisms. In this secretion they were first discovered; hence their name. The smegma bacilli are not alcohol-fast nor are all of those which are grown in culture media acid-fast. There is some evidence that the difficulty in decolorizing them by acids and the ease in decolorizing them with acid alcohol are due to a fatty coating of the secretion of the skin in which they thrive, and not to any quality of their own. Acid-fast organisms are found also in the nose, in the coating of the tongue, in the tonsillar crypts, in the stools, in the sputum, and in the lung, especially in cases of gangrene, but also in other cases. Besides these there are the organisms of tuberculosis of animals, birds, fish, reptiles. Of these organisms only those which attack cattle have received very careful study.

It is now agreed that the bacillus of human tuberculosis and that of bovine tuberculosis are not distinct species, although they do present certain differences. Both are pathogenic for man. The lesions of bovine tuberculosis in man involve the digestive rather than the respiratory tract. But this especially concerns pathologists. So far as the clinical microscopist is concerned, there is but one tubercle bacillus.

*Bacillus lepræ*, which resembles *Bacillus tuberculosis* in many particulars, is a slightly more slender organism. It is alcohol- and acid-fast. Some report that it can be cultivated (on media containing glycerine) with extreme difficulty. It is claimed that the bacillus obtained from cultures is not acid-fast. Some deny that any organism yet cultivated is *Bacillus lepræ*.

Since this organism is present in large numbers in the nasopharyngeal, urethral, and vaginal secretions, in the saliva, and in the feces, and, especially, since the nasal lesions are among the earliest of the disease, the search for, and the recognition of, this bacillus is certainly the duty of the clinical microscopist. Smears from these secretions are prepared and stained just as in the search

\* Burville-Holmes, Am. J. of Med. Sc., 1910, vol. 130, p. 99.

for tubercle bacilli, decolorizing them with acidified alcohol.

The non-pathogenic acid-fast bacilli certainly do not in any way constitute a biological group, and their ability to withstand decolorization by acid is about the only characteristic they have in common. While most of those found in our clinical examinations are shorter, thicker, and more homogeneously stained than the tubercle bacillus, yet some have exactly the same size and shape as that organism and morphology alone is by no means a sufficient criterion for the recognition of *Bacillus tuberculosis*. Practically none of the pseudo-tubercle bacilli resist decolorization by alcohol, and most of them are not very markedly acid-fast. We must admit, however, that the pseudo-tubercle bacilli other than the smegma are studied chiefly from cultures, while we seldom in clinical work study *Bacillus tuberculosis* in this way. We know that the smegma bacillus in culture loses some or all of its acid-fast reaction, and it is possible that should we obtain the other organisms in the same way in which we obtain the tubercle bacilli (*i.e.*, immediately from the host, without first growing them in cultures), they might be much more markedly acid-fast than we find them in cultures; but so far as we now know they all would be decolorized by alcohol.

Several <sup>21</sup> organisms of pseudo-tuberculosis have recently been described, but none which resemble *Bacillus tuberculosis*. Flexner described a streptothrix as cause of a condition in the lung resembling caseous pneumonia. Ophüles <sup>22</sup> reported five cases of gangrene due to long, slender, more or less curved rods or threads, with irregularly staining protoplasm, which frequently occur in clusters. In all cases it was possible to find individuals which showed true branching. These organisms were acid-fast (although not especially so), but all were decolorized by acid alcohol.

In some cases of pulmonary tuberculosis (cases which clinically are characteristic, and the diagnosis of which is confirmed by autopsy) no tubercle bacilli can be found in the sputum. Careful examination, however, of the sputum of these patients reveals small masses of granules which have all the staining characteristics of *Bacillus tuberculosis*. These granules are thought by several writers to have a diagnostic importance equal to that of the perfect tubercle bacillus.

In searching for tubercle bacilli, if the sputum be homogeneous it is well to study many specimens. Ten are recommended by some. In the technique of this clinic, using only selected particles, we consider it is necessary to examine but three. If we find none, we prefer to give a negative result and ask for another specimen, rather than search longer in that same sputum. Bacilli are most numerous in the muco-

<sup>21</sup> See Abbott and Gildersleeve, *Centralbl. f. Bakt.*, 1902, xxxi, p. 547.

<sup>22</sup> *Journ. of Med. Research*, 1902, iii, p. 242.

purulent or pure purulent sputum of cavities. They are very rare in the fibroid form of the disease, also in the caseous pneumonia before disintegration of the lung. One negative examination is valueless. Some will search for three days, others say six or seven, while in our cases we believe in searching as long as there is sputum, and in one case the bacilli were found only on the nineteenth examination, and Brown tells of one positive only on the twenty-sixth daily trial. All other methods failing, the inoculation into the guinea pig may be resorted to. "It is of doubtful value to put the sputum in the thermostat that the bacilli may grow."

The *prognostic value* of sputum examination.<sup>23</sup> When it is remembered that possibly many of the tubercle bacilli are not stained at all; that old foci may give off very few and young foci no bacilli at all, however actively they may be forming; that by the occlusion of the bronchus the contents of the focus may be shut off entirely for a time and when expelled the sputum contain a vast number of tubercle bacilli; that they may be present one day, then not again for months; that in the same specimen the organisms may be abundant in one part of the specimen and none in others; that some persons with fatal tuberculosis have no bacilli in the sputum, as, for instance, caseous pneumonia and acute miliary tuberculosis, while in other cases the bacilli are present even before the physical signs; lastly, that in the severe cases with bronchitis the secretion of the bronchi will dilute the sputum and give the appearance of a diminution in the bacilli, it will be seen that one must be very guarded in his use of the examination in forming an opinion of the prognosis. In general it may be said that while the examination of one specimen is of no value for this purpose, repeated examinations are of use. Brown recommends the application of a modified Gaffky's table in following a case (1/12 oil objective, II ocular used).

- I. Only 1 to 4 bacilli in whole preparation.
- II. Only 1 on an average in many fields.
- III. Only 1 on an average in each field.
- IV. 2 to 3 on an average in each field.
- V. 4 to 6 on an average in each field.
- VI. 7 to 12 on an average in each field.
- VII. 13 to 25 on an average in each field.
- VIII. About 50 on an average in each field.
- IX. About 100 on an average in each field.

Cases are thus classified and designated by the Roman numeral.

Among some of the general points it may be said that while no number, form, arrangement, or staining qualities of the organisms is of

<sup>23</sup> Brown, Montreal Med. Journ., October, 1901; Journ. Amer. Med. Assoc., February 21, 1903. The reader is referred to these articles, from which the most of the following paragraph is quoted.

absolute importance, the continued expectoration of large numbers would indicate a cavity; the sudden increase in their number, diffusely spread, very numerous, and an increase in the cellular elements, would indicate disintegration; the steady decrease lasting for some time would, if the physical signs also improve, indicate improvement; the case should be called "healed" only when the bacilli are absent for a long time. On the other hand, the continued presence of large numbers of bacilli does not of necessity indicate an active process, as, for instance, in Fowler's case, in which for fourteen years bacilli in large numbers were constantly present, and yet the case was in fair health and even improving. Such a patient, it is needless to say, is the source of the greatest danger to his neighbors, expectorating as he may from three to four billion bacilli each day. Trudeau has mentioned a similar case extending over a period of ten years.

Many have considered that the form of the bacillus is a more important sign than the numbers, the predominance of short rods indicating a rapid growth, while that of long a slower; yet both forms coexist. Brown considers that while in general morphology gives little or no aid, yet a predominance of short rods does indicate a more active process. Others claim that the arrangement is the important thing, that their presence in clumps and parallel groups indicates a lively growth, and groups of short bacilli a bad prognosis. Yet these clumps may be found in all cases, though more often in the severer. Bacilli which stain deeply are considered to possess an especially bad virulence. Yet the exceptions to all such rules are so numerous that they may be held only in a very broad way.

The question is often asked, Is the discovery of a single bacillus of importance? Attention should be called to the fact that these so-called "single bacilli" are often not bacilli at all. But supposing one single bacillus is found concerning which there is little doubt. This may have been deposited from the air, and other contaminating means are always possible, so the discovery should be confirmed on following days. With careful technique the presence of one bacillus is certainly important. On the other hand negative examinations do not necessarily exclude tuberculosis. In one case, it was only on the nineteenth examination that the bacilli were discovered.

A fairly accurate estimation of the number of bacilli may be made,<sup>24</sup> but for clinical purposes it is not worth the considerable trouble it requires.

SPUTUM FROM A CAVITY.—Some of the older writers (Winkel) have considered that the odor of the breath was of particular importance. The stagnant sputum from cavities has a heavy penetrating sweetish odor, and this gives its odor to the breath. In some cases the

<sup>24</sup> Nuttall, Johns Hopkins Hosp. Bull., May, 1891.

sputum from such a cavity in the cup will be odorless, while the breath is most offensive ( see page 22 ).

During cavity excavation the sputum is mucopurulent, expelled in masses which flatten in the cup to form coin-shaped clumps, the so-called "nummulæ." These are seen especially in the dark green or grass-green sputa of caseous pneumonia with cavity formation. They are green or dirty grayish-green in color, isolated, do not coalesce, and consist chiefly of pus; they sink at once in water; their odor is not bad. Some are full of small points, even millet-seed in size, containing much black pigment and elastic tissue, granular detritus, and few pus-cells. The cavity is full of such material. These are not, as was formerly supposed, characteristic of cavity, for masses macroscopically similar arise also in the larger bronchi. When softening is rapid the expectoration of 100 to 150 cc. a day of sputum is not rare. From large cavities most is expectorated in the morning. Blood is often present, which, if retained in the cavity, is expectorated in blackish clots. In case the cavity communicates with the bronchus by a fine hole, there may result the same skein of pus described under abscess of the lung. The sputum often has a sickening sweetish odor. In case bronchiectasis, gangrene, or putrid bronchitis occurs, with decomposition the odor may be foul, but it is remarkable how seldom these occur.

As the cavity clears and becomes lined with connective tissue, the character of the sputum changes considerably. We then have the "sputa globulosa," consisting of balls of a grayish-white color, thick, rounded, shaggy masses,—a conglomerate of mucus, detritus, and pus,—some of which sink in water, but not all. Large tissue fragments are rare unless the connective-tissue proliferation be rapid and dissects off particles of the necrotic cavity wall.

**HEMORRHAGE.**—This occurs in the majority of cases, in amounts varying from small flecks to cupfuls. In some cases the number of hemorrhages is so great they are termed "hæmoptysical" cases. Hemorrhage occurring early in the disease is very frequent, but seldom great, and recurs often. This is the so-called "inflammatory hemorrhage," seen especially at the onset of a caseous pneumonia or during acute exacerbations of the consolidation. It has the same significance as in acute lobar pneumonia, the blood escaping by diapedesis or from erosions of the mucosa. Later in the disease, however, the hemorrhages are of a very different character, since then profuse and sometimes fatal, occurring without warning in a person apparently recovering. Such arise from the rupture of the small miliary aneurisms in arteries which cross a cavity or are exposed in its wall.

**Croupous Pneumonia.**—In true lobar pneumonia very rarely there is no sputum at all, except in the case of very old or very young

patients. At the onset a hemorrhage is sometimes the initial symptom. In other cases the sputum is mucoid and abundant for even four or five days, but very soon becomes bloody, at first from the presence of unchanged red blood-cells. This sequence, mucoid then blood sputum, marks the progress of the inflammation from bronchi to alveoli. The sputum at this stage is remarkably transparent, since the cells are not present in rouleaux but are scattered singly throughout the mass. Soon, however, the sputum becomes rusty, is then characteristic in appearance, and when typical a diagnosis may be made from it alone, even when other signs fail. This rusty sputum is homogeneous, glairy, almost transparent, so tenacious and jelly-like that the cup can often be inverted without the loss of any. The color is due to the transformed hæmoglobin, and microscopically very few red blood-cells can be found. The above-described sputum is present only in cases in which there is not much catarrh of the larger bronchi, which furnishes mucopurulent masses. In amount it varies from about 150 to 300 cc. per day. When small in amount it dries rapidly in the cup, since there is so much albumin, so little mucus.

Blood is a quite constant feature of pneumonic sputum. For the most part uniformly distributed, it often is also present in streaks of varying size, while in other cases the sputum is almost pure blood. If the process extends to another part of the lung a rusty sputum may again become bloody.

In color it is typically of a rusty yellowish-brown hue, but in other cases with physical characteristics the same it is of an orange-yellow, a lemon-yellow, or a grass-green color; in fact, all the possible shades which are seen in subcutaneous bruises. These colors are due to different oxidation stages of unknown hæmoglobin derivatives (Traube). The sputum may appear jaundiced, but this term should never be used unless the skin is icteroid.

Microscopically is seen a transparent background with some red blood-cells which are swollen and pale as a rule, others well preserved; many epithelial cells, columnar or pavement; lymphocytes, granular cells, and oil globules. Chemically, this sputum is characterized by the absence of the alkaline phosphates, the excess of potassium over sodium, an increased amount of sulphates, and a large amount of soluble proteid. The fixed salts, usually about 18 per cent., are in these cases about 26 per cent.

At the crisis the sputum loses its rusty color and becomes mucopurulent, more or less abundant, and finally white mucus. "In no other (disease) is the cycle of sputa changes so marked or of so great diagnostic value as in this disease" (Mackenzie).

In addition to the study of the individual cases, a series of ninety-four were compiled to get some general idea of the relative frequency of the different forms



of sputum in our cases. Twenty-one per cent. of the cases denied having had any sputum at the onset of the disease; 46 per cent. denied that it was bloody, whereas 33 per cent. stated that the first sputum noticed was slightly bloody. During the course of the disease 16 per cent. of the cases had little or almost no sputum. One case was in the hospital seventeen days without any expectoration, and other cases about seven days. In 32 per cent. the sputum was typically rusty; in 39 per cent., not only rusty but blood-streaked; in 3 per cent., very bloody; while in 10 per cent. at no time during the disease was any blood noted.

VARIATIONS.—If bronchial catarrh be also present, that is, when a pneumonia supervenes on a chronic bronchitis (Traube), the sputum consists of mixed rusty pneumonic and mucopurulent bronchitic sputa. It is therefore quite fluid. It may not be rusty at all; it may be bloody mucoid pus. In some cases, instead of being rusty it is very bloody; *e.g.*, in the so-called "hæmorrhagic pneumonia" of the aged. In chronic passive congestion due to heart, lung, or renal disease, we have the characteristic "brick-red" sputum, thin like that of oedema, and very bloody. This is the sputum of "congestion" or "serous pneumonia" (Traube). It is seen when the inflammation proceeds by starts.

The *green sputa* are of particular importance: in cases of delayed crisis and lysis but in which perfect resolution may follow; in a case clinically becoming serious it is an important warning; it may be the first symptom of abscess of the lung, and should always arouse suspicion in a case with an abnormal course; in cases in which the skin is jaundiced it has no significance; and lastly and most important, it may be the first indication that the diagnosis of croupous pneumonia was incorrect, and a search should at once be begun for tubercle bacilli.

The sputum in the case of pneumonia which ends in necrosis or gangrene presents characteristic changes. It soon loses its tenacious and rusty character, becomes more fluid, its color changes from "rusty" to "coffee," then to "prune-juice," and later to "chocolate." The red blood-cells disappear. The odor, at first absent, is stale and later decidedly fetid. Granular detritus appears, and then necrotic fragments. Or it may throughout be prune-juice in nature, but this is rare. From these sputum changes the diagnosis can be made before the tissue fragments appear. The reason for the colors is not clear since the red blood-cells are described in some cases as well preserved.

The *prune-juice sputum* is also of particular importance. It usually indicates a severe type of the disease; in some cases, particularly in old persons, oedema of the lungs develops during a pneumonia,—and the rusty sputum becomes "prune-juice" in color; in other cases it indicates a low type of the disease; while still again, and in these cases without any serious significance, it merely signifies a beginning resolution.



*Fibrin coagula* are commonly found in a rusty sputum, as often, says Dr. Osler, as the search is made for them. Suspicious masses should be shaken out in water. These may be beautiful branching fibrin casts of varying size, the larger with hollow branches. In some very pretty casts there are found clots and small collections of blood in the lumen at each bifurcation of the branches. In one case the cast

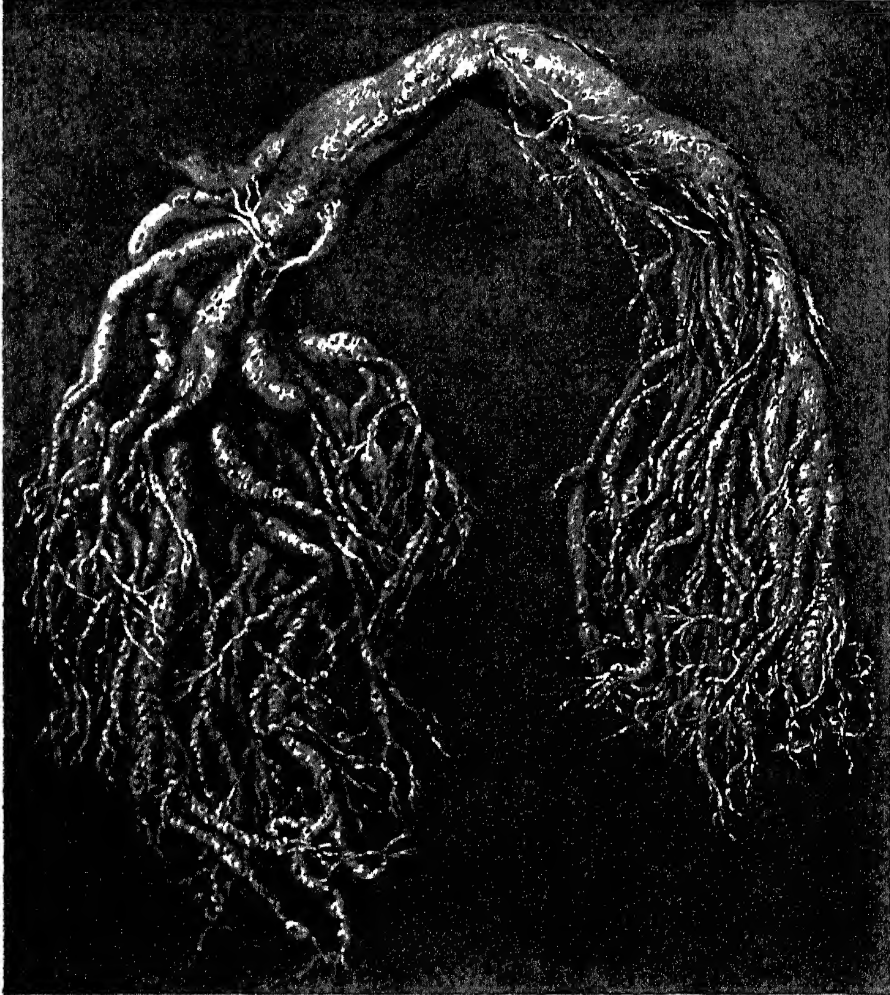


FIG. 15.—Fibrin cast from a case of double pneumonia. Natural size. The patient was a man 65 years of age. The cast was expectorated on the sixth day of a double pneumonia, followed by hemorrhage. Death on the seventh day.

seemed to be from two entire lobes, one of each lung, and hence to cross the bifurcation of the trachea. This cast is pictured in natural size as Fig. 15. *Curschmann spirals*, and, in fact, every constituent of asthma, may be found.

This was beautifully illustrated in Vierordt's case<sup>25</sup> of typical pneumonia but with intense general bronchitis and bloody sputum on the fourth and fifth days, with many fibrin coagula, which were particularly beautiful on the seventh day, at

<sup>25</sup> Berl. klin. Wochenschr., July 16, 1883.

which time also spirals were found and resolution began. From this time on were found beautiful Curschmann spirals, but no Charcot-Leyden crystals.

In the sputum of cases of acute lobar pneumonia will usually be found *Diplococcus pneumoniae* in large numbers. This is of great aid in the diagnosis of doubtful cases of lung trouble. Since this organism inhabits the mouths of about 50 per cent. of normal persons, although its presence there is better determined by animal inoculations and by cultures than by smear preparations made from the sputum, and since it is often found in small numbers in smears of the sputum in various other lung diseases, its demonstration is important only when it is present in large numbers. A thin film preparation of the sputum, or, to get a better smear, a small portion of sputum mixed with dilute serum, is made on a glass slide, dried in the air and then run slowly three times through a flame to fix the specimen. This is then stained with any bacterial stain, such as an aqueous solution of methylene blue; but it is important also to demonstrate the capsule and to use Gram's method (see page 89).

There is such a confusion of ideas as to the real value of the study of stained smears of sputum that a few words at this point may be appropriate. The student should be encouraged to make smears of the fresh sputum from the greatest variety of cases. He will find many of them full of organisms and will see that the number of these organisms increases rapidly as the sputum stands in a warm room. The cells full of biscuit-shaped diplococci especially will attract attention. The pneumococcus and bacteria resembling *Bacillus influenzae* will be found so often that one often doubts that there is any value at all in this examination. But if the sputum be expectorated into a sterile cup, the smears made immediately, and the question of abundance and not the presence of certain forms considered, this examination will be found of a little, although of very little, value.

*Diplococcus pneumoniae* is a small, oval coccus, about one micron in longest diameter, usually arranged in pairs, their short diameters parallel, but also often in chains. Even when in chains, one can usually see that the individuals are oval, their long diameters all in the line of the chain. The free ends of the diplococci are often pointed like a lancet (or better still, a candle flame), hence the name, "*Diplococcus lanceolatus*." *Diplococcus pneumoniae* is a capsulated organism whether in pairs or chains. There is always doubt about the identity of any non-capsulated diplococcus.

Among the capsule stains are the following. In all these stains the important point is to avoid the use of pure water.

*Welch's Method.*—The film, made in the manner described on page 50, air-dried and then passed through a flame slowly three times, is first covered with glacial acetic acid for five seconds. The

excess of the glacial acetic acid is then removed with filter paper and then washed off with aniline gentian violet (see page 89) until all the acetic acid is removed. The film is then washed in an 0.85 to 2 per cent. aqueous solution of sodium chloride until the stain is washed off. The specimen is examined at once in this fluid.

*Hiss's Potassium Carbonate Method.\**—The film preparation of sputum is dried in the air and passed through the flame in the usual manner. It is stained for a few seconds in a half-saturated aqueous solution of gentian violet. The dye is then washed off with a 0.25 per cent. aqueous solution of potassium carbonate, and the preparation studied in this fluid.

Very fair capsule stains may also be obtained by simply staining for about 30 seconds with the ordinary aqueous gentian violet solution (5 c.c. sat. alc. sol. to 95 c.c. distilled water), then washing with a 1 per cent. potassium carbonate solution and studying the specimen in this fluid.

*Hiss's Copper Sulphate Method for Permanent Mounts.*—The dried film is covered with a 5 per cent. or 10 per cent. aqueous solution of gentian violet or fuchsin (5 c.c. sat. alc. sol. to 95 c.c. distilled water), and held over a flame and allowed to steam for a few seconds. The staining fluid is then washed off with a 20 per cent. aqueous solution of copper sulphate. This solution is then poured off, and the specimen thoroughly dried between filter papers. When dry it is mounted in balsam.

*Buenger's Method.†*—Before the spread is completely dry it is covered with Zenker's fluid minus acetic acid (bichromate of potassium, 2.5 gm.; sodium sulphate, 1.0 gm.; water, 100 c.c.; bichloride of mercury till the fluid is saturated, *i.e.*, about 5 per cent.) and gently warmed over a small flame from 3 to 5 seconds. After this it is washed rapidly in water, flushed once or twice with alcohol (95 per cent.) and kept covered from 30 to 60 seconds or longer with tincture of iodine (about 7 per cent.). The specimen is next washed with alcohol (to remove the iodine) until the alcohol remains clear, when the specimen is dried in the air. It is then stained from 3 to 5 seconds with freshly prepared aniline-oil-gentian violet (aniline oil, 10; water, 100. This mixture is shaken and filtered, and to the filtrate are added 5 c.c. of saturated alcohol solution of gentian violet). The excess of stain is removed with a 2 per cent. NaCl solution. The spread is examined in this fluid.

\* Studies from the Dept. of Pathology of the College of Physicians and Surgeons, Columbia Univ., New York, 1904-1905, vol. x.

† Report of the Medical Commission for the Investigation of Acute Respiratory Diseases of the Dept. of Health of the City of New York; Part I., Studies on the Pneumococcus, 1905.

For methods of cultivation of *Diplococcus pneumoniae* the reader is referred to Buerger's article (*The Journal of Experimental Medicine*, 1905, vol. vii., No. 5). The student should always remember that by far the quickest and surest method of identifying this organism, is to inject a little of the culture medium, sputum, pleural fluid, or whatever specimen contains the organism in question into a mouse or rabbit. The animal will die in from 24 to 48 hours of septicæmia and in smears of the beast's blood can be found in goodly numbers these capsulated diplococci.

In the **subacute indurative pneumonia** the sputum may contain blood, but is seldom rusty. It is usually abundant, and there is a decided tendency for it later to become fetid.

**Chronic Interstitial Pneumonia.**—In this disease the cough is often paroxysmal, and in general the expectoration is copious, of a mucopurulent or a seropurulent nature, and sometimes fetid. Hemorrhage is present in about one-half of the cases.

**Bronchopneumonia.**—This term, which includes also the hypostatic, and the pneumonia of aspiration, is accompanied by an expectoration which combines the bronchitic with the pneumonic, that is, a mixture of rusty with mucopurulent sputum. Sometimes the transition from a bronchitis to a bronchopneumonia may be suspected from the changes in the sputum; it becomes less in amount, viscid, difficult to expel, and may be streaked with blood, but it is almost never typically rusty.

**Influenza.**—For some years it has been customary to distinguish between "influenza" and "grip." Grip was the term which designated all epidemic colds with the clinical symptoms of influenza, whatever might be the bacteriology of these cases, which was seldom determined. Influenza was any epidemic cold from which *Bacillus influenzae* of Pfeiffer could be isolated. Very soon, however, it was discovered that Pfeiffer's bacillus was an ubiquitous organism. To explain this its friends maintained that it persisted with diminished virulence in the area covered by an epidemic of influenza. Lord found it in 60 of 100 non-tuberculous cases with cough, and, in 29 of these, in pure culture. Recently the search for *Bacillus influenzae* has been undertaken in many clinics as a routine examination, and it has been found in a surprisingly large number of cases, about as many of which do not suggest influenza as do. Such cases are usually diagnosticated as chronic bronchitis, asthma, tuberculosis, etc. The duration of these cases had varied from months to years, and one probably had continued for forty-five years. *Bacillus influenzae* occurs in bronchopneumonia as a primary and as a secondary (*e.g.*, in diphtheria) invader. It is reported as a very common secondary invader in pertussis after the paroxysmal stage begins (Bordet's



FIG. 16. Section of influenza stained with Gram's and Biernacki brown, showing the *Bacillus influenzae* (brown), *Diphtheria* (blue), *et al.* (blue).  $\times 1000$ .



bacillus?), in the bronchitis of measles, in lobar pneumonia, and in tuberculosis with cavity. But it has also been reported as the primary cause of acute bronchiectasis, of cholecystitis, arthritis, pyelitis, cystitis, otitis media, acute nasal infections, empyema, meningitis, endocarditis, and has occasionally been reported as the organism of septicæmia. It would be strange if one organism could be the primary cause of so varied conditions, and Thursfield,\* among others, has emphasized the possibility that several quite different organisms are grouped under the term *Bacillus influenzae* because of a deceptive similarity in their morphological, and often in their cultural, characteristics. Not only has *Bacillus influenzae* been proved to be the cause of conditions which in no way resemble epidemic influenza, but recently even the most typical cases of these epidemics have been shown to be due to some other organism than this bacillus.

Now two opinions prevail. Some doubt whether Pfeiffer isolated the true cause of epidemic influenza, and think he found an organism which is a common secondary invader in various bronchial infections. Others believe that different epidemics of grip are due to different organisms, and that Pfeiffer did isolate the organism which caused the epidemic he studied. Davis,† for example, studied several epidemics which clinically were typical grip and believes that in one epidemic *Diplococcus pneumoniae* was the primary invading organism, in another *Streptococcus pyogenes*, in a third both of these were present. (That is, these were the organisms he found present in pure culture during the early acute stages of the attacks. Later in the attacks the infections were often found mixed.) He believes that these two organisms and *Streptococcus mucosus* are the chief causes of the serious complications and sequelæ of grip, and that *Bacillus influenzae*, while he found it very often late in the attacks, especially after the cough had become persistent, played little or no part in the primary etiology of these attacks, in their complications or in their sequelæ. He believes, however, that it did cause the epidemic of 1889-1890, and that Pfeiffer's observations during that epidemic were sufficiently confirmed.

*Bacillus influenzae* (see Fig. 16) is one of the smallest of the microscopically visible bacteria. It is a short, slender, non-motile bacillus, with rounded ends, and has a tendency to grow into filamentous forms. It stains faintly, and usually has a marked tendency to polar staining, so that it often resembles a diplococcus. It is decolorized by Gram's method and grows only on media containing hæmoglobin. The most profuse growth is obtained if pigeon's blood is used. Its growth and its virulence are increased if it is

\*Quart. Journ. of Med., Oct., 1910, vol. iv, No. 13.

†Arch. Int. Med., vol. ii, No. 2, p. 124.

grown with other organisms. For Lord's method of cultivating the organism the reader is referred to his paper. In the sputum it occurs chiefly in groups. Some of these groups are large and free, others are within leucocytes. (Some think the latter occurrence a sign of improvement.) *Bacillus influenzae* is sometimes virulent to animals (especially the guinea-pig and small rabbits), but often is not.

To recognize these bacilli in the sputum it is not so much a matter of stain as familiarity with their morphology.

A good method of staining this organism is as follows: the fixed smear is stained with the above-mentioned aniline oil gentian violet (Sterling's) for one and one-half minutes, and washed in water; covered with Gram's solution one and one-half minutes, and again washed in water; 95 per cent. alcohol, 5 minutes; wash in water; 0.2 per cent. aqueous Bismarck brown (twenty c.c. of saturated alcoholic solution of Bismarck brown diluted with 80 c.c. of water), one minute; wash, dry, mount.

The number of cases of so-called "influenza bronchopneumonia" receiving careful attention has been increasing rapidly since interest in the organisms of influenza was aroused. Davis \* found *Bacillus influenzae* the predominating organism in 10 per cent. of 26 cases studied, but in these cases it did not seem to be the primary invader. The sputum of patients with this disease is scanty at first, but later becomes profuse and purulent. Sometimes, although rarely, it contains traces of blood, which occurs in fine streaks and is not intimately mixed with the sputum, as in the rusty sputum of lobar pneumonia.

**Whooping-Cough.**—During the catarrhal stage the cough is, as a rule, that of a dry bronchitis. A little later the sputum of bronchitis presents no especial features, but during the paroxysmal stage the sputum is expectorated by very severe paroxysms of coughing in amounts very small each time, and yet in the aggregate considerable. Bordet's bacillus is now generally accepted as the cause of pertussis and is found early in whooping-cough in the tough masses of mucus, especially the shreds of mucus from the smallest bronchi, in pure culture. It is decolorized by Gram's method and shows marked polar staining. Morphologically it resembles *Bacillus influenzae*. Several have isolated a bacillus similar to Bordet's bacillus, but have not been able to differentiate it from *Bacillus influenzae*. Examined in the sputum, Bordet's bacillus is rather longer and plumper than the organism of influenza, "but as a result of cultivation it becomes smaller and smaller until finally it appears as a mere point under the highest powers of magnification" (Bordet). This organism does not grow on ordinary media, but on media which are weakly

\* Arch. Int. Med. vol. ii, No. 2, p. 124.



acid and poor in nutrient constituents, and which contain ascitic fluid or blood. The first growth is seen in two or three days. Bordet's bacillus can be trained to grow on media which do not contain blood. Bordet advises the following medium: One hundred grams of potato are cut into small slices and mixed with 200 c.c. of water containing 4 per cent. of glycerin and heated in an autoclave. The fluid is then decanted. To 50 c.c. of this extract of potato are added 150 c.c. of a 0.6 per cent. solution of sodium chloride and 5 grams of agar-agar. This is then autoclaved. While warm it is filtered into test-tubes, 2 or 3 cm. into each tube, and these tubes are sterilized. Blood (preferably human, although guinea-pig's blood will do), is defibrinated and is added to the agar in equal quantity in each tube. This is then shaken and slanted.

**Glanders of the Lung.**—In case the disease extends from the nose to the bronchi and there excites inflammation, the severe cough is said to be accompanied by a profuse purulent expectoration.

**Asthma.**—In acute bronchial asthma the sputum is perfectly characteristic, beginning, as a rule, only as the paroxysm begins to pass off, or, as the patient describes it, "breaks," and bringing with it much relief. During the paroxysm itself there is often no sputum; in other cases it is scanty, clear, consisting of thick glairy mucous balls, the so-called "perles of Laennec," which swim in a thin clear frothy mucus. In other cases it is less characteristic, of a greenish-yellow tenacious mucus, and described by the patient as "rubber-like." These perles are pellets of a semi-transparent mucus, of a pale gray color like boiled tapioca. In them are mucous moulds of the smaller tubes, and some on unravelling are Curschmann's spirals. Early the sputum contains a few eosinophilic cells and many alveolar epithelial cells with myelin degeneration. The moulds are small cylindrical or sausage-shaped masses consisting of thick threads, or plugs, which may be from 1 to 1.5 cm. long. Some branch, some are narrow or straight, while others are spiral. These have the same significance as the Curschmann spirals. The amount of sputum at this stage may be from very little to 50 cc. In the sputum also may be found alveolar cells with myelin degeneration and very few leucocytes.

In 27 per cent. of our cases there were in some of the paroxysms slight hemorrhages. As the attack "breaks," however, the sputum becomes thinner, more liquid, frothy, and much more abundant, even 200 cc. in twenty-four hours. It is then a clear viscid fluid in which float mucopurulent masses. In one of our cases was present a true bronchial cast about one and three-quarters inches long, consisting of mucus and eosinophile cells.

During the next two or three days the character of the sputum changes much. It is often small in amount and mucopurulent, with, however, some clear frothy fluid. As a rule, now no Curschmann's spirals are found, although in one case, in which they had been present in good numbers, they were more beautiful than

before. Fibrin casts of the bronchi are sometimes present, occurring with the spirals, which they may exceed in numbers. At the tip of some of the branches the cast may be continuous with the central fibre of a typical Curschmann spiral. Along with the spirals occur large numbers of eosinophile leucocytes. In some cases, but rarely, Mastzellen. Where the eosinophile cells are increased it is common also to find the Charcot-Leyden crystals. Calcium oxalate crystals have also been found. As a rule, the sputum ceases as soon as the attack is well over. In some cases, however, it is almost continuous, even 100 cc. per day, but may have sputum-free intervals.

**CURSCHMANN'S SPIRALS.**—These beautiful structures occur at some time in perhaps every case of true bronchial asthma. This, however, does not mean that they will be found in every paroxysm of this disease in which they are sought. We have in mind one case, a man whose sputum several years ago furnished the students with the most beautiful spirals. He has since then been admitted during the past fifteen years fourteen times in acute attacks of asthma. Only on one day of this period was a spiral found. While they may be present

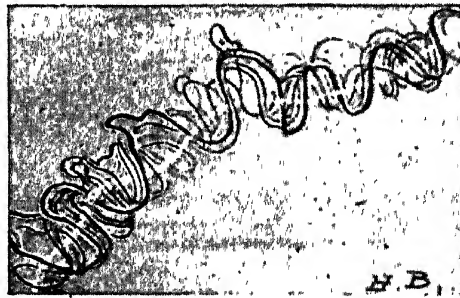


FIG. 17.—Curschmann's spiral, from the sputum of a case of asthma.  $\times 200$ .

during the paroxysm, they are found particularly just at the end, as the sputum increases, and, as a rule, are absent after it has become mucopurulent. They may be present in large numbers.

In general, two forms may be described. The first is a spirally twisted strand of mucus enclosing leucocytes, eosinophiles, and Charcot-Leyden crystals. The second and more beautiful form consists of a tight skein of mucus wound around a central fibre. This may be from 1 to 2 cm. or more long and 1 mm. broad. It may be branched. These spirals have two parts; the "mantle," which is the mucus surrounding the "central fibre." The mantle contains, besides eosinophiles, many pigmented epithelial cells, some ciliated cells, and Charcot-Leyden crystals. The arrangement of these cells, not mixed, but in lines and groups, is interesting. The central fibre, which probably consists of transformed mucus, is very refractive. It is a spirally twisted strand, homogeneous, with a sharp contour or saw-edged. While the caliber varies, it is quite constant throughout one spiral. Central fibres are subdivided into the small size, from 0.5 to 1 micron in diameter, the medium-sized, 3 microns, and the thickest, even 18

microns in diameter. In sputum which was hardened *en masse* and cut in sections they were found by Ruge to be solid without evidence of the lumen which others have claimed. These fibres are sometimes well developed, sometimes present only as a trace; they may be absent. They end sometimes as a thread, while in other spirals they give off a multitude of lateral threads. The finer are often branches of larger threads, or the smaller may unite to form a larger. Some of the larger types give off fine threads radially to the mantle in all directions. In structure some are lamellated, while others seem to be a bundle of parallel threads, spirally twisted. These central fibres may be differentiated by staining, and hence are not optical phenomena presented by the most compressed part of the spiral, as some claim. They occur alone sometimes (see Fig. 18), and have the same significance as per-

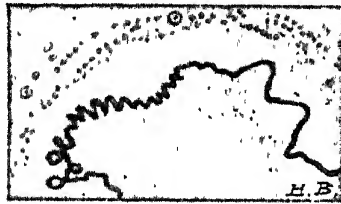


FIG. 18.—Free central fibre of a Curschmann's spiral, from the sputum of a case of asthma.  $\times 200$ .

fect spirals. When alone they are spirally twisted. In some cases occur perfect spirals; in others, many free central fibres; again others, free central fibres, and some with very imperfect mantles (see Fig. 17).

As regards the origin of the spirals, the central fibres are certainly not casts of the smaller bronchi, since they are often only about one-tenth as big. Schmidt claimed that for their formation the epithelium must be well preserved, that they consisted of mucus secreted in the smaller bronchi, the centre representing the most twisted part, that is, of the greatest relative compactness; and for their formation a tough mucus was the important thing. Hoffmann claimed that the smaller bronchi are themselves spirals, which become straightened out as the lung expands, and that the tough mucus forced through these spirals can assume a spiral shape. Others claim that the cilia motion in the bronchi must be in spiral waves; others that the spiral is formed from a straight band of mucus which in passing the bifurcation of two bronchi is whipped into a spiral by the cilia of the other bronchus, the direction of whose cilia motion will be tangential to the axis of this thread. Gerlach gave three conditions necessary for their production,—a small amount of very viscid sputum, very forcible respiratory movements, and clear bronchi. These three conditions are best given in asthma. He claimed that the mantle and central fibre are formed in the same place, but that the latter is formed later and is merely an optical expression for that part of the mucous mass which has been twisted the most. We would say, however, from studying the spirals we have seen, that while the central fibre is itself spirally twisted, there are many fewer revolutions per unit of length than in the mantle.

In cases which we recently had a chance to study the spirals were beautiful, about 2.5 cm. long and 1 mm. broad, and with a central thread so refractive that it could be definitely seen by the naked eye. While fresh it is interesting that this thread could not be studied with the higher powers because of its high refractivity,

and seemed to be merely an optical phenomenon. At certain points, however, where the mantle was thin and particularly after the spirals were allowed to dry somewhat, it could be easily studied, and was found in some cases to be a bundle of twisted fibres. The mantle was very tightly twisted, and on cross-section at certain points it could be seen that it consisted of spirally wound sheets of mucus reaching from the central thread to the periphery. It was filled with various cells; among these, squamous epithelial cells, very many alveolar cells, many containing coal pigment, and others modified hæmoglobin. In all fields were a great number of eosinophiles, in many, cylindrical cells which appeared ciliated, although the cilia were not as distinct as might be desired, and among them goblet-cells. It is interesting that in the structure of the spiral these cells were not mingled, but each variety occurred in groups or lines and large numbers in each group. The Charcot-Leyden crystals occur singly or in clumps, some quite large. One was projecting from an eosinophile cell and surrounded by its detritus. In some fields full of leucocytes search was made in vain for one which was not an eosinophile. In one specimen was found a strip of mucosa of cylindrical epithelium. Other spirals had no core, and some spirals consisted merely of a very large refractile central fibre. One spiral was particularly interesting; two separate strands of cell-rich mucus, discrete at first, became twisted, the one within the other, into a spiral, and yet each could be traced separately for a time until the coil was so tight they could not be distinguished. The inner was thick, the outer thin, and at the end where they were not spirally twisted their structure could be seen, a band of mucus with detritus and cells in lines; almost all the leucocytes were eosinophilic. If the spirals be allowed to dry, the central thread is much more distinct. In some cases they are a bundle of longitudinal fibres slightly twisted. This case was a typical one, with spirals, immense numbers of eosinophile cells, and a large number of epithelial cells, the alveolar with the various forms of pigment, and the cylindrical, both ciliated and goblet.

In another case were many central fibres without mantles, and some with a few fibres wound around them. These fibres, forming these imperfect, loose mantles, were remarkably thread-like and of quite uniform diameter.

**CHARCOT-LEYDEN CRYSTALS.**—These Charcot-Leyden crystals (see page 34), present wherever the eosinophile cells are increased, are very common in asthmatic sputum. Their number may greatly increase as the sputum stands. These crystals occur in groups, forming specks of a greenish-yellow color, which masses may be even seen with the naked eye. They may give to the spiral a yellowish-green color macroscopically when present in large clumps along the spiral. It is important to bear in mind that their size varies so that their presence can be excluded only when a search has been made with the oil-immersion lens. They increase in size and number as the attack lasts. In cases in which none were found at first they will appear in the sputum if it be kept in a warm chamber. Concerning their composition all that can be said is that there exists in the sputum a substance which after expectoration is crystallizable in this form. The same is true of tyrosin, and the French seem eager to identify the two. They practically always occur in asthma, but careful search is necessary, and in case they are not found it is well to let the sputum stand.

The **ALVEOLAR EPITHELIAL CELLS** laden with golden-yellow pigment may occur in large numbers and fill a considerable part of the mantle of some of the spirals. They occur chiefly in clumps packed

together, and in other parts of the specimen none will be found. It is interesting to see what large masses of these cells will occur in certain parts of the spirals. Their origin, v. Noorden says, is clear; that in asthma we have frequently small traces of blood in the sputum, and that this is the source of the pigment of these phagocytes. These cells are similar to the Herzfehlerzellen seen in chronic passive congestion.

There is a well-marked group of cases which may present a transitional stage between asthma and fibrinous bronchitis. The sputum contains spirals, Charcot-Leyden crystals, eosinophile cells, all the constituents found in asthma, but also casts of the smaller bronchi, which, however, do not branch much and which may at one end tail off into the central fibre of a true spiral. In one case of Dr. Osler's,<sup>28</sup> already mentioned, these casts were 1 to 3 cm. long.

**Acute Bronchitis.**—The sputum in acute bronchitis is at the onset very scanty, or even absent. When present this "sputum crudum" is usually tenacious, viscid, very hard to expectorate, and of a frothy transparent appearance. It consists of almost pure mucin. Microscopically a few leucocytes and red blood-cells can be found, also a few bronchial epithelial cells, some ciliated and some with the cilia in motion. There are also a few mononuclear leucocytes, the so-called "mucous corpuscles" which are derived from the lymphatic masses along the tract. In certain conditions so many of these epithelial cells are present that the term "desquamatory bronchial catarrh" was applied. Myelin drops are present, but they are not very numerous and in only the simpler forms. Such sputum is the result of an increased secretion of the mucous glands, together with the desquamation of a few epithelial cells. As a rule, this clear sputum is present for about two days, yet during the whole course of the acute bronchitis the sputum may represent only a hypersecretion, and hence present the above character. In some of our cases it was two weeks before very much pus was present. In some cases the sputum at this stage is less viscid, hence Biermer's adjective, "seromucous."

After the first two days or more the cough usually "loosens." The sputum is increased in amount, less viscid and less tenacious, and may be like the white of an egg in appearance, since it is frothy and shows whitish streaks. Sometimes it is blood-streaked, while in other cases there is considerable blood at the beginning of the attack; the latter was true in 33 per cent. of our cases.

The sputum now becomes mucopurulent. It contains all of the above elements, but the pus-cells are very much increased. These may be uniformly distributed, and give the sputum a uniform yellow color, or they may be present in purulent islands. There are still many epithelial cells present, but these have lost their shape and their cilia, are now

\* See Bettmann, Amer. Jour. Med. Sci., February, 1902.

round, and often fatty. Such sputum was formerly called "sputum coctum." In a typical case the sputum then becomes almost purulent, the pus being poured from the inflamed and probably partially denuded mucosa. This sputum is opaque yellow or a yellowish-green, and is often expectorated in masses. The amount is, as a rule, from 100 to 200 cc. in twenty-four hours. Most is expectorated in the morning. Microscopically, it is found to contain much mucus and much myelin. There are no cylindrical cells now. There are usually some red blood-corpuscles, but the leucocytes predominate in the field, and are chiefly polymorphonuclears, although a certain number of mononuclears are found. Alveolar epithelial cells, some containing pigment and some fat granules, may be found if searched for. Fat is also present in larger masses, which in shape resemble a cell, although now neither nucleus nor protoplasm can be demonstrated. The sputum of some cases is characterized by the abundance of fat, present in cells, in droplets, and in the above-mentioned masses of droplets, while in other cases but little is found. The reason for this difference is not known (Hoffmann). Bierman divided the purulent sputa into three classes. His division has been severely criticised, although the nummular variety was present in a few of our cases. With improvement the sputum becomes more abundant, more purulent, and less tenacious. It then, as improvement continues, diminishes in amount and finally disappears. The above is a quite typical sequence. The following varieties, however, occur. In 13 per cent. of our cases in which the diagnosis of acute bronchitis was made because of the physical signs on auscultation, no sputum was at any time to be obtained. In some cases so tenacious was it that it could not be expectorated, the patient often vomiting in the attempt. In other cases the sputum is mucopurulent and fairly abundant from the very onset. It is interesting, however, that in a large number of these last cases it is probable there was a slight chronic bronchitis already present, since over 50 per cent. of these patients stated in their history that they were subject to coughs and colds. This was true in less than 20 per cent. of those in which the sputum at the onset was of small amount. In general it seems true that a large amount of sputum means a chronic trouble. In about 35 per cent. of our cases the sputum was viscid and very tenacious and scanty throughout the whole course, the patient suffering from a dry cough for a few days after the disappearance of the sputum. Many cases have at the end of the attack a common sputum, not mucopurulent, but consisting of a watery serum in which swim islands of pus which are globules about 1 cm. in diameter, consisting of mucus loaded with pus-cells. Such a sputum on standing will separate into two layers, the upper watery and transparent, the lower purulent. This was true in 10 per cent. of our cases. Other cases were interesting in



that the sputum at the end of the attack became again as at the beginning of a pure mucous type. In acute bronchitis much valuable information may be obtained from the sputum, since it is the best index of that which occurs within the chest.

In the so-called *capillary bronchitis*, that is, acute bronchitis of the smaller tubes, the cough is frequent, often paroxysmal, and at first dry. It may remain so, the sputum being absent throughout the entire course, or expectorated in small quantities with great difficulty. In these cases a diminution in viscosity is a sign of improvement.

**CHEMICAL ANALYSIS.**—The chemistry of the sputum in this disease has a very slight interest. Of the cases which have been reported by Bamberger, Biermer, and Renk, the water has varied from 95.62 to 98.3 per cent.; the organic substances, from 1.17 to 3.7 per cent., while the inorganic, from 0.457 to 0.76 per cent.

**Chronic Bronchitis.**—Under this heading may be included all cases from the simple subacute, in which case a cough has merely “held on” for several months, to those cases which give a history of slight cough with expectoration extending over twenty-five or more years. Among the cases which may be considered subacute we have those in which for several weeks or months the sputum is tenacious, viscid, and very small in amount. The patients describe this as consisting of thick leathery lumps; in other cases, as a white sticky mucus. Later on it is apt to become more and more abundant and mucopurulent, and hence yellower. Some sputa have a dark greenish color and a foul odor which will last for weeks. Such sputum as exemplified in our cases is abundant, and will separate to a certain degree into three layers,—a mucous layer, brownish-gray serum, and a mucopurulent sediment. The sputum in these cases will gradually diminish leaving the patient apparently well, but certainly more susceptible to another acute attack.

The acute exacerbations of a very chronic bronchitis form no small part of the admissions for acute bronchitis in a general hospital. These exacerbations may turn a dry cough to one with sputum, or a chronic expectoration of slimy mucus to an abundant mucopurulent sputum, often blood-streaked.

During the acute exacerbations it varies much in appearance. Sometimes it is small in amount, very tenacious and purulent, sometimes of large amount, mucopurulent and slightly tenacious, and in still other cases, and perhaps most common, is an abundant white frothy seromucous sputum containing very little pus. The odor is sometimes foul, and in one of our cases almost putrid. The amount may vary from 100 to 200 cc. in twenty-four hours. Later the sputum increases in amount and presents mucopurulent flakes, sometimes very small. It separates into two layers, with the serum above and the solid particles below, while other cases will have a tenacious green mucus upper layer and a fluid lower layer. Microscopically are found pus, epithelium, and red blood-cells. The most common type of chronic bronchitis is the so-called winter cough, the patient during the winter suffering from cough and expectoration from which he is free during the summer.

This may be the history for fully twenty years. Later, however, the tendency is for the troubles to be continuous throughout the year. Such cases, as a rule, expectorate only in the morning, and describe themselves as then "clear" for the day. Some such cases expectorate about an ounce of mucopurulent sputum, while in others it is in thick yellowish masses. In severe cases the cough is paroxysmal and the sputum a sticky, frothy phlegm, sometimes blood-streaked, and very hard to expectorate. During the acute exacerbations it is apt to become still more scanty and tenacious. In general, these patients feel best when the sputum is moderate in amount, and worse if diminished or increased. Microscopically, it contains much mucus, few pus-cells, except in the purulent variety, and much myelin. In some cases it is profuse, mucopurulent, the pus being present in nummular masses of a yellowish-green color which float in a liquid serum, thus causing it to separate into two layers. In other cases it may also be very large in amount but homogeneous and extremely viscid, filling the cup with a single purulent glutinous jelly-like mass.

**DRY CATARRH.**—The "catarrhe sec." in the sense of Laennec, is a disputed symptom-complex, but a chronic bronchitis with very little or no sputum is not at all unusual. According to the English, this occurs particularly in "gouty" patients. We associate it, however, more with emphysema and myocarditis. Some of these cases will deny any sputum whatever; in other cases it is glutinous and pearly.

**THE CHRONIC BRONCHITIS OF EMPHYSEMA** deserves especial mention, since it is such a common form. For instance, of 100 of our cases of chronic bronchitis, in 43 per cent. the emphysema was a marked clinical feature. Of 100 cases of emphysema 58 per cent. suffered also from bronchitis, and 47 per cent. from chronic bronchitis. Of the cases with chronic bronchitis, in 11 per cent. it was the dry form, the patient denying any expectoration whatever. In the cases with a slight sputum the expectoration occurred for years only in the morning, and for the most part consisted of a slight amount of bluish-white mucus. It may, however, be large in amount. One case, for instance, for years was awakened at five o'clock each morning with a severe paroxysm of coughing and expectorated large amounts of thick mucus, in an almost solid mass. In other cases the sputum is abundant, whitish in color, frothy, and amounts to one pint a day. In our cases of emphysema with chronic bronchitis and admitted during an acute exacerbation of the bronchitis the changes in the sputum, due to the acute exacerbation, were very varied. As a rule the amount increased very materially. In some cases it was very gelatinous, in some a white frothy mucus, in some a blood-stained serum, and in one case it became putrid. In one-fifth of the cases it was blood-streaked. As the cases improved it first became still more abundant, white and frothy, and then gradually diminished to the previous state. Among these sputa of chronic bronchitis a few may be mentioned in particular. In two a great many eosinophile cells were present. Some were remarkable because of the large amount of myelin, and in others large masses of fat globules were very noticeable. In one case of simple chronic bronchitis the man had had for ten or twelve years a slight expectoration. On admission the sputum was thin, cloudy, and abundant, consisting of serum in which was a sediment containing moulds of the bronchi from medium size to 0.5 mm. in diameter, and consisting of mucus with pus and alveolar epithelial cells. The sputum also contained much pigmented alveolar epithelium, pus-cells, and red blood-cells. In a case of chronic bronchitis in an emphysematous person during a rather acute attack with a steadily elevated temperature the sputum was found to be considerable in amount, seromucoid, and never bloody in appearance, and contained during repeated examinations large numbers of sarcinae. In one case of chronic bronchitis with emphysema and "hay fever" the sputum was yellowish-green, mucopurulent, slightly blood-tinged, containing branched plugs



of the bronchi which consisted of mucus and pus in which were many eosinophile cells and masses of the mycelial threads of some mould.

In the chronic bronchitis of cardiac disease, especially mitral, the sputum is characterized by the large amount of blood which may be present. This may be fresh or changed enough to give a prune-juice appearance, and in cases, particularly of mitral stenosis, may be grossly stained by the large numbers of *Herzfehlerzellen* which are constantly present. In other cases there is a daily large amount of frothy seromucous pus.

**BRONCHORRHOEA.**—If by bronchorrhœa one means, with Laennec, a chronic idiopathic disease, the existence of this form is exceedingly doubtful. But if by the term is meant a chronic bronchitis with an abundant sputum, it is by no means rare. There is one form described, no example of which we have had in this hospital, of a “bronchorrhœa serosa,” or “asthma humidum,” with an abundant, very watery, colorless, foamy sputum. Some of these cases are said to have a neurotic basis. In the bronchorrhœa of chronic bronchitis the sputum may be very large in amount; commonly it is purulent, watery, of a green or a yellowish-green color, and in amount about 500 cc. a day. In these cases of “bronchoblennorrhœa” the bronchi have been denuded of mucosa and are lined by a pyogenic membrane, hence little mucus is secreted and the sputum is a profuse watery pus which separates easily in three layers and which may have a very bad odor, although not distinctly fetid. Such sputum is seen also in bronchiectasis, and perhaps in cases of putrid bronchitis and lung gangrene.

**PUTRID BRONCHITIS.**—In some cases of chronic bronchitis there is a disagreeable almost fetid odor to the sputum, but in putrid bronchitis a truly fetid expectoration is present. This occurs with most cases of bronchiectasis, gangrene of the lung, abscess, those in which the sputum decomposes within tuberculous cavities, and in empyema perforating through the lung. A true simple bronchitis without dilated tubules and yet with a fetid expectoration is certainly very rare (Fowler and Godley), while some deny that it ever exists (Hoffmann), and claim that the most of the cases thus catalogued are probably of bronchiectasis. A case of putrid bronchitis would quite surely result soon in dilatation of the bronchi, and a case of bronchiectasis very often soon has a fetid expectoration. A very few genuine cases have, however, come to autopsy (Osler).

The sputum is an abundant, profuse, watery pus, of a dirty ashy-gray or a brownish color, and with a horrible odor which sometimes will fill the whole house. Allowed to stand, it separates into three layers,—the upper of frothy air-containing mucus, usually small in amount, since the mucous membrane is for the most part destroyed and replaced by a pyogenic membrane, and hence secretes little mucus;

from this layer extend downward brownish strands. The middle layer is of serum, while the lowest is a thick sediment of epithelial cells, fatty cells, free fat, almost pure pus, all kinds of bacteria, and sometimes Dittrich's plugs. No elastic tissue or fragments of lung are to be found, thus excluding gangrene. Gangrene, however, may follow. Chemically are found many of the products of decomposition of proteids, volatile acids, among them butyric, valeric, and others;  $\text{NH}_3$ ,  $\text{H}_2\text{S}$ , leucin, tyrosin, etc.

FIBRINOUS, CROUPOUS, OR PLASTIC BRONCHITIS.—By this term we here mean the chronic, idiopathic form, not the acute form occurring in the course of certain infectious fevers (see page 24). This chronic form is a very rare disease as is shown by the fact that Bettmann was able to find only twenty-seven cases in the literature of thirty-five years. This disease is very little understood. The sputum is for about five or ten days catarrhal, consisting of abundant mucus, and then after a severe coughing spell a bronchial cast is expectorated. Blood is quite often present in the sputum, either before or after the expectoration of the cast, but generally with it, and yet true hemorrhage is rare. The frequency with which casts are expectorated varies much. Usually months intervene; but in some cases a cast is expectorated every two or three days, or even every day, and in one case three were expectorated in one day. They are seen as formless masses in the sputum. After shaking them out in water they are found to be moulds of a bronchial tree. Those from the same case will often present exactly the same shape as if they were all from the same lobe. Sometimes they will appear to represent the tree of a whole lobe. The size of the largest of them is about 10 cm. long. They are grayish-white in color, contain a great many air-bubbles, and in about one-third of the cases are blood-streaked or contain a clot in the centre. On cross-section they are found to consist of concentric layers, apparent either grossly or microscopically; the inner layer presents many whorls, since this layer, the oldest, has been telescoped into those more recently formed. The casts are usually hollow, although some are solid; others are hollow in the larger branches and solid in the smaller, and still others *vice versa*. In the central layer, the oldest, are seen the remains of many cells, alveolar and bronchial epithelium, leucocytes, red blood-cells and bacteria. Sometimes there is much fat in the casts and in the sputum.

Casts do not always arise from nor are they produced by the epithelial cells of the mucosa, since in the above-mentioned case in which three were expectorated in one day, there was found to be no epithelium in that part of the bronchial tree. These were therefore a direct exudation. They were formerly supposed to consist of fibrin, since physically the material resembled this. Others claim that they are of

mucus, one says syntonin, another coagulated albumin, because of the chemical reactions. Some portions take Weigert's fibrin stain, but the most of it does not, hence it may be said that their composition is rather uncertain. Liebermeister<sup>29</sup> reviews the question at length as the result of the study of one fresh case and twelve museum specimens. He found fibrin and mucin present in seven of the thirteen cases. Weigert's fibrin stain cannot be trusted in these cases. For fibrin he prefers Kockel's method, and thionin for the mucin. In fibrinous bronchitis the cast is of a loose texture containing much air, almost fills the lumen, and contains few cells. In diphtheria of the bronchi the cast consists of a firm hollow membrane of dense fibrin strands with countless cells. A cast from a heart case at death was similar to those of fibrinous bronchitis.

Charcot-Leyden crystals are commonly present in the cast. In the same sputum sometimes spirals are found. In Dr. Osler's case, mentioned by Bettman, the ends of some branches of the casts were directly continuous with the central threads of true spirals. Many eosinophile cells are sometimes found, also red blood-cells, hæmatoidin crystals, and lecithin granules. In Vierordt's case<sup>30</sup> there were many such casts, and on one occasion a typical Curschmann spiral.

**Bronchiectasis.**—The sputum in the saccular form of this disease is often very characteristic; in the diffuse form not at all. In the former it is marked by two features,—its profuseness and the periodicity with which it is expectorated. This periodic feature was well shown in ten of our twenty-four cases. The expectoration occurs usually in the morning, and depends upon the position of the sac; an irritation of the bronchus due to a discharge of some of the contents of the sac caused by a change in attitude leads to a paroxysm of coughing and hence the emptying of the whole sac. The amount is profuse, as a rule, from 750 to 900 cc. in twenty-four hours, while in one of our cases it frequently exceeded one litre. Such profuse expectoration may extend over a considerable period of time.

In general it may be said that the amount bears no relation to the duration of the disease, for one case of twenty-six years' standing expectorated but from 15 to 30 cc. a day. Nor does it bear any relation to the size of the cavity, as was shown by one of our patients who expectorated more than one litre of sputum a day and yet at autopsy a few surprisingly small cavities were found. Of twenty-three cases, in two the sputum for twenty-four hours was under 100 cc.; in eleven, from 1 to 300; in two, about 500; while in seven, over 600 cc. It is stated that the diminution in amount as the patient grows weak before death is surprising.

The most characteristic sputum is grayish or grayish-brown in color, fluid, purulent, of a disagreeable odor, and separates into three

<sup>29</sup> Deutsch. Arch. f. klin. Med., 1904, Bd. 80, 5 and 6.

<sup>30</sup> Berl. klin. Wochenschr., July 16, 1883.

layers on standing. This character, however, is by no means constant. A bronchiectatic cavity lined by mucous membrane will before infection secrete a pure clear mucus, but after infection has occurred, as is the rule, the mucosa is soon reduced to a pyogenic membrane which secretes a yellow purulent fluid with a sweetish odor. This may last for years. Sooner or later putrefactive changes may set in. The sputum is then mucopurulent, and of any shade of gray or green; those with the worst odor in our cases were of a dirty gray color. If blood be present the color will present different shades of red or brown according to the chemical changes in the hæmoglobin. While as a rule it is very fluid and watery, in some cases it is thick and viscid, while in other cases the sputum is mucopurulent and contains masses suggesting nummulæ. In other cases, as was shown in our series, particularly those improving under treatment, while it was profuse and watery at first, it later diminished in amount, was mucopurulent, and of a less offensive odor. The tendency to form three layers on standing in a tall glass vessel was marked in fourteen cases. These layers are: an upper frothy mucous layer, a middle serous, and a lower granular layer. From the upper often hang down through the fluid strands or "streamers," as they are sometimes called, of the same material. Hoffmann and others mention but two layers, omitting the upper mucous, which was absent in three of our cases. The lowest layer is always thick.

In four of our cases there were four well-marked layers; the lowest of an abundant, greenish-red, purulent material; the one above containing a good deal of blood, and hence was red or brown; over this a serous layer, while on top a frothy mucous layer. In other cases below the frothy mucous layer was a mucopurulent layer with streamers hanging down through the fluid and which with a little encouragement would probably all have sunk. The odor is, in general, bad, but in two of our cases it was not at all offensive. In some cases there is at first none, then a slightly offensive odor of a heavy, sweet nature, while after the putrefactive changes have set in it will be of a fetid character. These changes are due to secondary infections of the contents of the cavity. In ten of our cases the odor was heavy and sweet, while in ten others it was at some time very fetid. This is not exactly the same odor as in gangrene, but has been described in some cases as "pseudo-gangrenous," resembling the odor of rotten cabbage, or garlic. This odor will often diminish after creosote inhalations, or intratracheal injections, and a patient admitted with extremely fetid sputum may leave the hospital with a much reduced sputum not at all offensive. The breath is sometimes worse than the sputum. The odor is largely due to  $H_2S$ ,  $NH_3$ , and various volatile acids, among which are acetic, butyric, and formic.

Hemorrhages into the cavity are common. Some put the figure at 50 per cent. They occurred in seventeen of our twenty-four cases. While slight, as a rule (eight cases), it is sometimes considerable (six cases) while in three of our cases it was extreme. In other cases it is fatal.

One of our cases, a man, was admitted to the hospital fourteen times, and five times because of extreme hemorrhage which threatened his life. At one of these admissions, in the course of a very few days he had six large and several small hemorrhages, reducing his blood rapidly from about normal to 1,090,000 red blood-cells with 20 per cent. of hæmoglobin. In another case on one day 1700 cc. of blood were lost in about ten minutes. Another similar hemorrhage the next day was fatal.

**MICROSCOPICAL CONSTITUENTS.**—As long as a bronchiectatic cavity is uninfected it will contain mucus and desquamated epithelium cells. After infection its wall becomes a pyogenic membrane and its contents those of an abscess. Later, infection with organisms of putrefaction is the rule. The pus-cells, enormous in numbers, are well preserved, fatty, or vacuolated. The red blood-cells are unchanged or very much altered. Elastic tissue indicates ulceration of the walls and was present in two of our cases. The fatty acid crystals occur especially when the outlet of the cavity is small, thus allowing considerable stagnation. These crystals are often very large in size, numerous, and present a beautiful picture. They are abundant in four of our cases (see Fig. 6). Cholesterin occurs; hæmatoidin crystals, leucin, and tyrosin, sometimes; Dittrich's plugs very commonly. The alveolar epithelial cells are usually present, containing pigment and, in some cases, much myelin or fat. No tubercle bacilli are found, but bacteria in great numbers in large zooglœa. Yeasts occur, and in one of our cases a definite aspergillus mould was found. In the contents of these cavities calcium salts are sometimes deposited, giving rise either to a clay-like mass or to the so-called "lung-stones." In two of our cases these lung-stones were present, and in one of them, that of a man who had expectorated several, at autopsy considerable calcareous concretion was found embedded in the walls of a cavity. The stones in the sputum were about the size of a split pea.

In other cases, as in thirteen of our series, the sputum is by no means so characteristic, but presents all the characteristics of a chronic or a fetid bronchitis.

Children with bronchiectasis are apt to swallow and then vomit their sputum.

**Gangrene of the Lung.**—In this disease the sputum most characteristic is profuse, extremely fetid in odor, of a greenish-brown color, separates easily into layers, and contains shreds of tissue. The latter point alone differentiates it from putrid bronchitis. Its odor is the worst of all, yet in some there is none whatever and no fetor of the breath. In five of our twelve cases the presence of gangrene was unsuspected, one case expectorating merely "phlegm." This odorless sputum is seen particularly in diabetics (in one of our cases the gangrene was an autopsy surprise), and in the insane. In cases of embolism the infarcted area may become gangrenous and be discharged through a bronchus. It is often difficult to differentiate between gangrene

and abscess of the lung, since whichever is primary the other is quite sure to develop. The odor of the sputum will not be of great assistance in a differential diagnosis between gangrene and long-standing bronchiectasis; for a positive diagnosis of gangrene one must find tissue fragments.

The sputum of a case of pulmonary gangrene is profuse, watery, and usually of a dirty greenish-brown or ashy-gray color. But the color of those which contain blood will vary from reddish-brown to brownish-red according to the degree to which the hæmoglobin is changed. Other sputa are described as chocolate in color. The sputum separates easily into three layers,—the upper of frothy mucus, the middle of serum, and the lowest, always a large one, of pus, tissue detritus, Dittrich's plugs, and tissue fragments. From the top layer streamers often extend down through the fluid. In other cases the sputum is mucopurulent in nature. It may be viscid, lumpy, mixed with blood, and yet very fetid.

Macroscopically of chief interest are the fragments of necrotic lung tissue. These may be minute or fragments several centimetres long. They are often sooty in appearance, with ragged outline. It is in this disease that the very large fragments of lung tissue are expectorated. These fragments are of firm tissue, or of colorless ground substance full of granular detritus or fat droplets, clumps of coal, large fat needles, bacteria, and elastic tissue. Dittrich's plugs are also found. The other constituents of the sputum are those of fetid bronchitis. There has been considerable dispute as to whether the presence of elastic tissue has any diagnostic importance. Some claim that it is rarely if ever present in this disease, and think it is digested by a ferment. Osler says that he has never seen a case in which it was absent. To be of aid in diagnosis the elastic tissue must show clearly alveolar arrangement. In bronchiectasis with ulceration of the walls of the cavities fibres of elastic tissue may be found in the sputum. Alveolar epithelium, often pigmented, occurs; fatty acid crystals and fat droplets are abundant; cholesterin, leucin, tyrosin may be present; masses of bacteria and of leptothrix occur, while flagellata have been described. In one case mentioned by Sahli, in which the infected area was non-odorous, large numbers of sarcinæ were found. Blood is frequently present, and in large amounts. These hemorrhages are principally from small vessels, not from diapedesis. Fresh blood was present in five of our cases, but as a rule it is much altered and the hæmoglobin present as methæmoglobin and hæmatin.

Many observers have found acid-resisting organisms in the sputum of such cases (see page 51). Mayer found them in ten of fifty-eight cases. They are not alcohol-fast.

**Abscess of the Lung.**—In abscess of the lung the most characteristic feature of the sputum is the sudden appearance of a large amount



of quite pure pus in which are fragments of lung tissue. It may be many hundred cubic centimetres in amount. If allowed to stand it will present a certain layer formation, but not a characteristic one, since a slight shaking will restore its previous homogeneity. Its odor is at first faintly sweet like all pus, but when gangrene supervenes, as it often does, it becomes foul, yet less foul than that of the average case of gangrene and putrid bronchitis. The lung-tissue fragments are of particular importance. They are permeated by pus-cells which give them a yellowish-gray color. In size they vary from about a millet-seed to fragments even two inches long. They consist of a framework of elastic tissue, the remains of blood-vessels, masses of coal-dust, fat crystals, free fat, detritus, hæmatoidin crystals, amorphous clumps of pigment, and zoogloea of cocci. In other cases there is a so-called "insensible disintegration" (Leyden) of the lung without the appearance of any large fragments. In such cases separate elastic fibres will be found. The other microscopical constituents are free elastic fibres, cholesterin, fatty acid crystals, free fat, lung pigment, detritus, bacteria, and hæmatoidin crystals, which may be present in large numbers and give to the whole mass of sputum a brown color. In the text-books, particularly of older writers, has been described the gross appearance of a sputum which escapes from a large cavity slowly through a small opening. In this case the pus as it escapes in a thin thread receives a mucous coating which prevents its coalescence. Hence the sputum when shaken out in water will appear like a skein or thread of pus. In our cases there has been no such appearance.

The sputum of a LIVER ABSCESS perforating through the lung is often characteristic. This sometimes causes also a lung abscess, in other cases an hepaticobronchial fistula without a local abscess. The sputum may have a so-called "anchovy-sauce" appearance, or the tint may be ochre-yellow due to the bile. The patient will complain of the bitter taste due to the bile acids. Microscopically bilirubin crystals and much elastic tissue will be found.

In our records there is a series of seven such cases. In three the sputum was abundant, exceeding even a litre in twenty-four hours. Expectoration may be paroxysmal, even a quart at a time. The odor was mildly offensive in two, and markedly so in two others. In six of these cases the sputum presented the typical anchovy-sauce appearance; that is, it was of a rusty brownish-red color, and frothy. In four cases it was blood-streaked, and in two purulent. Microscopically may be found the ordinary elements of sputum, pus, red blood-cells and alveolar epithelial cells. In addition the hæmatoidin (bilirubin) crystals or needles may be a marked feature, as was true of two cases. Elastic tissue was found in considerable amounts in five cases, and at times was in considerable quantity. Fat crystals were present. In two cases the liver cells, it was thought, could be recognized. The living active amœbæ were found in five cases, in one long before they could be found in the stools even after repeated examinations. It is interesting to note how often the sputum which contains the amœbæ will also contain much elastic tissue. If the sputum be preserved in the thermostat, they will remain alive and motile for a day or so.

**ABSCCESS OF THE LUNG FOLLOWING ACUTE LOBAR PNEUMONIA.**—In three of six cases there were no clinical features which would suggest this discovery at autopsy. In these cases the abscesses were small and multiple, and there was little or no sputum. In one case in which the diagnosis was not made the only change in the sputum was that the viscid tenacious blood-streaked expectoration became less tenacious. In one case a small amount of a very tenacious blood-tinged sputum became suddenly very dark, of a brownish-black color, mucopurulent, and then greenish and small in amount. It then disappeared, soon to reappear as a mucopurulent, very green, scanty sputum, and soon became large in amount, very thick, very purulent, and of a sour odor. It then became thinner, more watery, but blood-stained, containing elastic tissue. Then it reduced in amount, became mucopurulent, and finally, with the recovery of the case, ceased. In one case large numbers of trichomonads were in the sputum.

Three cases of **POST-OPERATIVE ABSCESS** were followed clinically; one was admitted with a paroxysmal cough and the sudden expectoration of a foul-tasting sputum, which later became sweetish and of a less disagreeable odor [yet bad enough we thought]. The expectoration was large in amount, and contained pus and fatty acid crystals. There were large fragments evidently of tissue, even 5 by 3 cm. in size, but so decomposed that the structure could not be well made out. The sputum then became less profuse, mucopurulent, and the patient recovered. In another case the sputum was very foul and contained much fat, while in the last case it was large in amount, foul, purulent, and blood-streaked. Of two other cases, in one the sputum did increase, but the diagnosis was not made, while in the other an abundant, blood-streaked, brownish sputum of no especial odor suddenly increased in amount, became dirty, frothy, and foul, slightly streaked with blood, and separated easily into three layers. At autopsy a large abscess cavity was found.

**Perforating Empyema.**—The sputum of these cases resembles abscess of the lung, with the exception that there is less elastic tissue and practically no tissue fragments. There will be many hæmatoidin and other crystals. The odor, that of pus at first, in some cases described as resembling old cheese, is soon vile because of the infection which commonly follows. In case the pleural fluid escapes slowly through a small opening, it is said that there may be present the fibrillary nature of the pus seen when an abscess is discharged, and due to a coating of mucus around the thread of pus. When the opening is large the pus will escape often rapidly, yet without causing pneumothorax. Allowed to stand, it separates into three layers,—the upper of mucus, the middle of the pus serum, and the lowest of pus-cells.

**Perforating Serous Pleurisy.**—This is exceedingly rare. The sputum is like that of oedema of the lungs, but contains more albumin, becoming even solid on boiling.

**The Serous Sputum of Oedema of the Lungs.**—In these cases there are expectorated large amounts of a frothy, cloudy, colorless, or a slightly bloody sputum which on standing separates into three layers: an upper abundant frothy layer, a foamy fluid, and a lower thin layer of pus together with the elements of the pre-existing sputum. Excepting in cases of pneumonia, etc., it is largely quite pure serum, which is frothy since it is so rich in albumin, watery, since directly from the



blood, and contains only a trace of mucin. Patients with this sputum flowing in streams from their nostrils and mouth during their last hours of life are one of the saddest and most gruesome sights of the wards.

**The Albuminous Expectoration of Thoracentesis.**—Among the recent articles on this subject to which the student is referred are those of Riesman<sup>31</sup> and Allen.<sup>32</sup> This sputum usually appears after a thoracentesis in which the fluid was withdrawn either too rapidly or too completely. Terrilon has grouped the cases into three classes. The first is of mild cases, the sputum varying from little in amount to 800 c.c.; the condition of these patients is always good. The severe cases have dyspnoea and collapse, and expectorate from 1200 to 1500 c.c. The grave cases begin suddenly; the fluid may gush from the mouth. The patient may die at once from suffocation from the fluid which he cannot expectorate rapidly enough, and, indeed, he may die before he can expectorate any.

The onset is, as a rule, in less than one hour, or it may come on during aspiration. The latest case began eighteen hours after the tapping, and lasted for twenty-four hours. The duration may be from several hours to a day, but as a rule it is from one to two hours. The fluid is richly albuminous and hence viscid, frothy, and neutral or faintly alkaline in reaction. Chemically it may be tested by heat and nitric acid, or by nitric acid alone, or by potassium ferrocyanide. The sputum should be diluted and filtered and the filtrate tested. Acetic acid gives a precipitate of mucin. It also contains urea, hæmoglobin, and the various salts of blood-serum. Urobilin has been found. The amount is generally from 200 to 900 cc. Two litres have been expectorated. On standing it separates into three layers,—the upper whitish and frothy, the middle opalescent and yellowish or greenish, the lower more viscid, containing a few whitish flocculi, and sometimes slight traces of blood, but rarely much. In Riesman's case there was no lower layer, the specific gravity was 1.018, the fluid became solid on heating, the total solids were 5.84 per cent. In Allen's case reported from this clinic the expectoration began in half an hour after 3100 cc. of pleural fluid had been removed, and lasted four hours. It was about one litre in amount, frothy, pale green in color, with a muddy sediment. Microscopically were found flat epithelial cells, a few leucocytes and red blood-cells, and many bacteria. The analyses differ widely. The fluid, while sometimes resembling that of the pleural exudate, in some analyses differs considerably from it. The cause has been much disputed. The majority of writers think that it is due to an acute œdema of the lungs, and is the result of their too

<sup>31</sup> Amer. Jour. Med. Sci., April, 1902, p. 620.

<sup>32</sup> Johns Hopkins Hosp. Bull., January, 1903.

rapid expansion, but the mechanism of which is very much disputed. We would call attention, however, to certain cases occurring during parathoracentesis and followed by pneumothorax. Some of these cases suggest the expectoration of the pleural exudate, and the demonstration that in many cases the two fluids differ does not disprove the claim that in certain the fluid does come from the pleural cavity.

**Hæmoptysis.**—For the causes of pulmonary hemorrhage we will give a summary of the chapter on this subject in Osler's text-book.

Hæmoptysis may occur (1) in young healthy persons without known cause and without subsequent symptoms. (2) As the first symptom of pulmonary tuberculosis, or (3) in a well-marked case. During the early stages it is due to mucous erosions and diapedesis; later to the rupture of an aneurism in a branch of the pulmonary artery, which is exposed by cavity formation. (4) Other diseases of the lungs, and this list includes practically all pulmonary disease. Among them are pneumonia at the onset, "bloody bronchitis," cancer, gangrene, abscess, bronchiectasis, tumors, cysts, and actinomycosis. (5) Heart disease, especially mitral. As a rule slight, yet it may be profuse and recur for years. (6) Vascular degeneration, the result of increased pulmonary tension, seen in emphysema and arteriosclerosis. (7) In ulcerations of the larynx, trachea, and bronchi it may be profuse and rapidly fatal. (8) In aneurisms it is sometimes sudden and fatal; in other cases the so-called "weeping" may persist for weeks, or the pressure of the aneurism as a tumor may cause an erosion of the mucosa. (9) An extremely rare form of vicarious hemorrhage due to interrupted menstruation. (10) In rheumatism. (11) Malignant fevers, the so-called hemorrhagic type. (12) Purpura hæmorrhagica and various other blood diseases, among which are hæmophilia, leukæmia, and scurvy. (13) Distomatosis (Westermanii).

The amount of the blood may vary from a mere speck or a few small clots to a quart or more. In general it is of a bright red color even when of venous origin since it is aërated in the lungs, frothy from its admixture with air, and always coughed up. When it clots in the bronchi, casts of these may be formed. In gastric hemorrhage, as a rule the blood is dark, due to the transformed hæmoglobin the result of the action of the acid gastric juice, not frothy, partly coagulated and vomited. Such points are easy enough to determine when the doctor is the observer, but from the history given often difficult, for in their anxiety the friends will not notice such fine points. The severe coughing often causes vomiting, while the coughed blood may be swallowed and vomited. Aspiration of blood from a gastric ulcer will also cause a certain amount of coughing. The gastric blood may be bright if the stomach be empty and a large artery be opened, while the pulmonary blood may be dark and not frothy, provided a

large branch of a pulmonary artery be eroded. It is most important in order to determine the origin of the blood to ascertain whether there has been previous lung or stomach trouble. Following a pulmonary hemorrhage the sputum will for some days be blood-tinged, while after a hemorrhage from the stomach there is usually considerable blood in the stools, but during hæmoptysis some of the blood is swallowed and this may be found in the stools. It is important to recognize the so-called spurious hæmoptysis, in which cases the blood may arise from varicosities of the veins at the back of the tongue or lesions in the throat, glottis, or œsophagus. It is said to be common for young anæmic girls to find blood on their pillows in the morning which has oozed from their spongy gums.

**Hemorrhagic Infarction.**—In many cases this diagnosis may be made from the inspection of the sputum alone. This is expectorated in masses which remain discrete in the cup, and which appear to be of pure blood, but are found to consist of a very tenacious mucus intimately mixed with pure fresh blood. In other cases these balls consist of glairy mucus streaked with blood. Expectoration begins at once with the cough and the pain, and the character of the previous sputum changes considerably at this time. Such was true of half of our cases. Microscopically, the mucus and the red blood-cells form the most of the mass, and leucocytes are remarkably few in number or even absent, while alveolar cells loaded with blood pigment are usually present in enormous numbers. This, however, may be explained from the fact that these infarctions are particularly common in mitral disease. In other cases the sputum is much less characteristic. This is true if there had been considerable sputum previous to the embolism. Sometimes there is a real hemorrhage, as in one-third of our cases; sometimes the sputum is pneumonic in character; in other cases it resembles the brick-red sputum of chronic passive congestion, and in these the diagnosis is said to be hard. In one-fifth of our cases there was practically no sputum. (In one, however, there may have been some blood-streaked sputum before admission to the hospital.)

The distinctive characteristics of the sputum of these patients are soon lost, sometimes in a very few days. Usually in about one week the sputum is merely blood-stained and will soon be free from blood. With recovery also it becomes more watery. The amount of blood certainly bears no relation to the size of the infarctions. This was well seen in one of our cases with very large infarctions and only slightly blood-streaked sputum.

**Chronic Passive Congestion.**—In chronic passive congestion, especially due to mitral disease and particularly stenosis, the sputum is characteristic. The expectoration is chiefly in the early morning, and

consists of a white mucous background, colored by dots or streaks of a rusty color; or the whole mass may be uniformly rusty. These dots, streaks, or uniform tinting are due to the large masses of Herzfehlerzellen; that is, to the alveolar epithelial cells laden with golden yellow granules of amorphous pigment derived from the red blood-cells which have escaped into the alveoli by diapedesis. It is in this condition that the large number and the constant presence of these cells have a great diagnostic importance. This importance was impressed upon us by one case which I will mention in detail. The man spoke only a language for which we could obtain no interpreter; a history of his case was therefore out of the question. His heart was repeatedly examined and reported practically negative. The sputum, however, contained constantly large numbers of Herzfehlerzellen. The pleural exudate was hæmorrhagic and contained mulberry-like masses of the proliferated endothelium of the pleural cavity. He died in a few days without a diagnosis. At autopsy there was found a mitral stenosis of an extreme degree, one of those cases common enough without any heart murmurs, and several large pulmonary infarctions.

**Malignant Disease of the Lungs.**—The sputum of this has in some cases been described as “characteristically gelatinous, of a red or blackish-red color like currant-jelly,” but this is by no means common. In other more common cases it has a prune-juice character. A grass-green or an olive-green sputum has also been found resembling that of caseous pneumonia. A prune-juice sputum (present in ten of eighteen cases) Stokes thought an important sign. In any case a search should always be made for the fragments of the tumor. Our cases presented no important points. In one case of secondary metastasis into the lung, although the area involved was large, there was practically no sputum. In another case of a large tumor the sputum was very viscid, slightly rusty, of a greenish-red color, not fetid, and consisted of pus, red blood-cells, and alveolar epithelium with much myelin degeneration. The next day it was of a dirty grayish mucopurulent character, and at times contained considerable blood. In the case of an epithelioma of the bronchus there was considerable expectoration and several severe hemorrhages. At other times the sputum was seropurulent, liquid, blood-streaked, not tenacious, and frothy. Diagnosis has in several cases been made from the tissue fragments in the sputum.

In mediastinal growths the expectoration is due to the bronchitis resulting from the pressure, and will present the various characters of this condition. If, however, the size of the tumor causes a narrowing of the bronchus, this may lead to bronchiectatic cavities, and a profuse fetid expectoration be the result. Gangrene may supervene.

**Syphilis of the Lung.**—Fowler and Godley state: "Evidence of excavation with fetid expectoration which does not contain tubercle bacilli should always suggest the possibility of the case being one of pulmonary lues." The expectoration may be profuse, purulent, and offensive, fetor being a common characteristic in advanced cases. With stenosis of the bronchus, a common event in this disease due to the extensive formation of connective tissue at the hilum of the lung, bronchiectatic cavities will form and the sputum present all of the characters of this condition. While hemorrhage is not common, some cases attract attention by the remarkably bloody nature of the sputum. Some writers state that unless repeated examinations for the tubercle bacilli be made, these cases will pass for consumption. Osler, on the other hand, states that he has never seen a case which resembled tuberculosis clinically.

**Pneumoconiosis.**—According to the dust which is inhaled this condition has received various names,—anthracosis, if it is coal-dust; siderosis, if iron dust; and chalicosis, in which it is a silicate or other rock-dust. The expectoration is in general mucopurulent, often profuse, and laden with the above-mentioned dusts (see page 21).

**Diphtheria.**—Cultures should be made from the throat of all those patients concerning whom the question arises whether they have or have had diphtheria. Cultures should certainly be made if there is any membrane visible, but also if the throat of one known to have been exposed to diphtheria shows a follicular tonsillitis, or even if only congested. If a person known to have been exposed has any constitutional symptoms suggesting infection, the bacteriological examination of the throat should be made even though it appears perfectly normal. Examinations should be made at frequent intervals after an attack of diphtheria until two successive examinations fail to demonstrate this bacillus. Also the nasal secretions of all persons with chronic coryza who have been exposed to diphtheria should be examined. Cultures should be made from any membrane forming on a superficial wound in the skin or on any mucous membrane. The reason for these careful examinations is not nearly so much for the sake of the patient as for the safety of his neighbors.

Cultures and smears are best made from fragments of the membrane itself. If a shred cannot be picked from the surface with a pair of forceps, material for the culture can be obtained on a swab made up of a wad of cotton wrapped on the end of a stiff wire about eight inches in length. This is put into a test tube, cotton end in, the free end sticking out, the tube closed with a cotton plug and then sterilized. Cultures should not be made from the throat within two hours after an antiseptic gargle has been used.

This sterile cotton swab is forcibly rubbed against the edge of the

patch of membrane when this is visible, and when there is no membrane present, against any exudate or over any injected area. Smears are then made from fragments of membrane or from the swab, and the swab is then rubbed forcibly and thoroughly over the moist surface of solidified blood serum. Failures to cultivate the bacillus are frequently due to the fact that the swab was rubbed over the centre of the patch of membrane, or not forcibly enough, and that the surface of the serum was too dry, or that the swab was not sufficiently rubbed against the serum.

The serum tube is put into the thermostat as soon as possible and left there at 37° C. for from eight to twenty hours. Smears are then made from the growth.

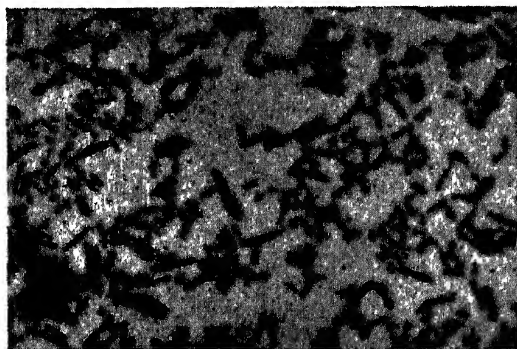


FIG. 19.—*Bacillus diphtheriæ*, from a young blood serum culture.  
Photomicrograph by Dr. Thomas M. Wright.

*Bacillus diphtheriæ*, or the Klebs-Löffler bacillus, is a small straight or slightly curved rod, from one to six, average two or three microns in length. It is a non-motile, non-liquefying, non-spore-producing aërobe. It grows on all ordinary culture media, providing they are not acid nor too alkaline, but best on blood serum.

One of the best media is *Löffler's blood serum*. This is a mixture of blood serum three parts, bouillon containing one per cent. of glucose, two parts. It is coagulated at about 70°C. When no tubes of media are at hand, cultures can be made on the coagulated white of a hard boiled egg. The shell is lifted at one end of the egg, the surface inoculated and the shell put back. The egg is then put in a thermostat.

*Bacillus diphtheriæ* grows with great rapidity on the above mentioned serum. At the end of even eight hours in some cases the growth can be seen and the organisms easily found in smears, but a negative examination at that time has no value. In any case the growth can be determined in 18 or 20 hours. Until this time the diphtheria bacillus has dominated, and smears from the surface of the serum look like pure cultures. After this time, however, the organisms from the throat may begin to dominate and soon crowd out the

diphtheria bacillus, unless the latter was present in pure culture in the throat. The colonies of *Bacillus diphtheriæ* on blood serum are moderate in size, elevated, of a grayish-white color and with opaque centres.

This organism when properly stained presents an almost characteristic appearance. Smear preparations for microscopic examination are made by scraping some of the growth from the surface of the serum with a platinum needle. This is then rubbed on a glass slide, allowed to dry in the air, and the slide passed through a flame three times, in order to fix the specimen.

A good stain is *Löffler's Methylene Blue* (saturated alcoholic solution of methylene blue 30 cc., aqueous solution of potassium hydrate, 1:10,000, 100 cc.). The slide is covered with this stain and warmed for a few minutes (it will not overstain), the stain is then washed off in running water and the specimen dried with blotting paper. The specimen can be improved by washing it further with 0.1 per cent. acetic acid, but this is seldom necessary.

Bacilli stained in this way show at their ends or along their bodies deeply staining granules called "polar granules" which appear as deep blue dots. In many there are several such granules, giving the organism a beaded appearance.

*Neisser's* stain is supposed to differentiate this organism from all others and is adopted as the standard in clinical work. The specimen is stained for about five minutes with a methylene blue solution. (Methylene blue, Grüber, 1 gm., 96 per cent. alcohol 20 cc., distilled water 950 cc., glacial acetic acid 50 cc.) This stain is filtered before using. During the staining the dye should be frequently renewed and the specimen gently heated. The specimen is then washed in water. It is next stained in Bismarck brown solution for two minutes. (Bismarck brown 2 gm. dissolved in 1000 cc. of distilled water.) The pole granules are stained a deep blue and the bodies of the bacilli a light brown color.

*Gram stain.*—The smear is stained for one and a half minutes with aniline-gentian-violet. (Saturated alcoholic solution of gentian violet 5 cc., aniline water 100 cc. The aniline water is made by slowly mixing aniline oil 1 part with distilled water 20 parts. The mixture is allowed to stand for some hours and then filtered until clear.) It is then washed in water, and then put for one or two minutes in Gram's or Lugol's Iodine solution. (Iodine 1 part, potassium iodide 2 parts, water 300 parts.) The specimen is then washed in absolute alcohol for three to five minutes. It is then mounted. Or it may be counterstained with Bismarck brown, washed and dried, and mounted. Bacilli which "stain by Gram," or are "Gram positive" retain the purplish hue derived from the gentian violet. Those which "decolorize by Gram" or are "Gram negative" will appear unstained unless a counter-stain is used, in which case they take the color of the counter-stain.

*Bacillus diphtheriæ* is characterized, in addition to its manner of growth already mentioned, by its irregularity in staining, and its irregularity in size and shape. Its irregularity in staining is shown by its polar granules or beaded appearance, but this depends in part on the age of the growth and may entirely fail. These bacilli vary much in size. Some recognize a "long" form and a "short" form and think these forms differ in virulence. But no relation between



length and virulence has been determined. The length seems to depend rather on the stage of the disease when the culture is made and the age of the growth.

For the clinical laboratory examination, smears are made directly from the throat and blood serum is inoculated. The growth on the serum is examined in less than twenty-four hours. From these two sets of smears, those directly from the throat and those from the serum, both stained by Neisser's method, the diagnosis is made. The smears from the swab may show diphtheria bacilli and no growth be obtained, and frequently the growth will succeed when the search over the smears was negative.

One looks especially for bacilli about five microns long with brown bodies and two blue staining polar granules, one at each end, in the presence of which a positive diagnosis of diphtheria may always be made. Hosts of other shapes and forms may be seen, but the presence of this form decides the question. The barred and beaded forms are not as common as these with the two polar granules, which are present in most localities in over 90 per cent. of the cases. If the initial culture be older than twenty-four hours, these forms may not be seen. One may then find the host of involution forms with their bizarre shapes: spindle, pear, dumb-bell, lancet-club forms and the varicosed forms. These might be found in the smears made immediately from the throat, but they could not be told from involution forms of other mouth organisms.

If the organism be cultivated for several generations it rapidly loses its characteristic morphology and can then not be accurately differentiated from other bacilli. For these reasons the clinical laboratory diagnosis of diphtheria is made from the examination of the smears made directly from the throat and those from the twenty-four hour, or younger, culture. If the typical forms are not found in these, it is better to make a fresh bacteriological examination than to cultivate the original culture further.

In addition to *Bacillus diphtheriæ*, one often finds in the throat *Micrococcus aureus* and *Streptococcus pyogenes*.

The value of the bacteriological examination of the throat is emphasized by the fact that McCollum was able to grow the organism from the throats of 40 per cent. of 500 cases whose throat condition suggested, but was not characteristic of, diphtheria, and that in many instances positive cultures were obtained from 24 to 48 hours before any membrane appeared.

The statement is often made that *Bacillus diphtheriæ* can be found in the throats of healthy persons who have not been exposed to diphtheria. McCollum states that Löffler found them in 4 of 160, Park and Beebe in 8 of 330, Kober in 5 of 600, Denny in 1 of 235, and



he in none of 130 such persons examined. He also doubts that it is often found in the throats of healthy persons who have been exposed to this disease, since he failed to find it in any of 60 nurses from the diphtheria wards of the Boston City Hospital. While it is possible that some of the earlier reports were made before the question of pseudo-diphtheria bacilli had arisen, it is undoubtedly true that the *Bacillus diphtheriæ* can live for a long time in the throats and noses of those who have recovered from an attack of diphtheria.

The test of its virulence is often important for the recognition of the diphtheria bacillus. A guinea pig is inoculated subcutaneously with a bouillon culture of the organism in question. This culture should not be over 48 hours old, since its virulence tends to diminish after that time. The amount injected should equal 1 per cent. of the animal's weight. If the organism injected be *Bacillus diphtheriæ*, the animal will show symptoms of acute or of chronic infection. If the infection be acute, the animal will die in from one to six days. At the seat of inoculation will be found extensive necrosis with a marked inflammatory reaction. There will be extensive œdema of the abdominal wall, effusions into the serous cavities, hemorrhages into the adrenals, swelling of and hemorrhages into the lymph glands, and focal necroses in the various organs.

If the injection cause a chronic infection, the animal will show paralysis similar to that in man, and will die in about six weeks.

Formerly all organisms with the morphology described above were considered *Bacillus diphtheriæ*. Since then various pseudo-diphtheria bacilli have been described, and the relation of these to *Bacillus diphtheriæ* is still a mooted question in bacteriology.

The following groups of organisms may be mentioned:

1. Bacilli with the typical morphology, typical cultural characteristics, especially the ability to form acid from glucose, and which produce the typical lesions in animals, are in the opinion of all observers *Bacillus diphtheriæ*.

2. Bacilli with typical morphology, and typical cultural reactions, especially the ability to form acid from glucose, but which are not pathogenic to animals, may be called avirulent diphtheria bacilli. Roux maintains, however, that the ability of the organism to ferment the sugars is not an essential characteristic of the species.

3. Bacilli with typical morphology, but which do not conform in their cultural reaction with the diphtheria bacillus and which are either non-pathogenic to animals, or do not produce typical lesions, may properly be called pseudo-diphtheria bacilli.

4. Finally, there are a number of organisms which resemble *Bacillus diphtheriæ* in many ways, but whose morphology is not

exactly the same, since they do not show bipolar staining and are often shorter and a little thicker than the typical form, and which have different cultural characteristics and differ in their pathogenicity. This group certainly includes the pseudo-diphtheria bacillus of Hoffmann, the xerosis bacillus, and others.

But for the clinical laboratory worker this classification has little interest. He should work with fresh smears and cultures less than 24 hours old. If he calls all organisms with a characteristic morphology *Bacillus diphtheriæ*, his mistakes will number less than one in a hundred. This odd case will not be hurt if treated for diphtheria, and the community will be safer if no chances are taken.

VINCENT'S ANGINA, "Plaut's angina," "ulceromembranous stomatitis," and "ulceromembranous angina," are some of the names applied to acute or subacute febrile infections of the tonsils or mouth, characterized by the formation of deep penetrating ulcers often covered by a pseudomembrane, and the presence in smears made from the base of these ulcers of large numbers of certain bacteria.

The base of the ulcer is mopped with a sterile cotton swab and smears at once made, dried, run through the flame three times, and stained with carbolfuchsin.

*Carbolfuchsin*.—Basic fuchsin 1 part, absolute alcohol 10 parts, 5 per cent. carbolic acid, 100 parts. The specimen may be covered with the concentrated stain for about half a minute, or, better, this stain diluted with from five to ten times its volume of water and then left on for about five minutes.



FIG. 20.—Smear from the throat of Dr. Louis P. Hamburger's case of Vincent's angina. Photomicrograph by Dr. H. Schapiro.

In typical specimens the field will be found crowded with various cocci and bacilli, and especially with *Bacillus fusiformis* and a spirochæte. *Bacillus fusiformis* (Vincent) is a long slender bacillus often fusiform in shape, the ends being quite pointed. It is straight as a rule, but many are curved, and a few may be S-shaped. It is non-motile (disputed), decolorizes by Gram (disputed), is often beaded, and can be grown in pure culture (Weaver and Tunnicliff, *Jour. of Infect. Dis.*, 1905, ii. p. 446).

This organism has been found in many normal mouths, on the surface of normal tonsils, in the cavities of decayed teeth, in the exudate of pyorrhœa alveolaris, in antrum disease, aphthous ulcers, but also in fetid abscesses almost anywhere in the body. The organism is common enough but has escaped notice since it is with difficulty grown, and when seen in smears is usually passed by as a "harmless saprophyte." Recent work is rather in favor of the view that this organism is pyogenic.

In the mouth this organism is often but not always associated with a spirillum or spirochæte, which is from 15 to 25 microns long, shows from two to five spiral turns, is actively motile, is Gram negative, and stains so faintly that it is often overlooked. It has not been cultivated. This is quite certainly a saprophyte which occurs in enormous numbers in the mouths of even healthy persons, and yet it is so frequently associated with *Bacillus fusiformis* that the two are supposed to be symbiotic and together to cause the ulcers.

These few lines concerning Vincent's angina are not inserted so much because of any importance of the disease as a separate disease, for this question is a disputed one, but rather to emphasize the importance of the student's studying the flora of the mouth. The number of organisms there is great—over one hundred different forms have been found. Among them are those which are so constant that they are considered the natural mouth-flora, bacilli, spirilla, and various leptothrix and spirochæte forms, many of them huge, many showing grotesque involution forms, and all with one common characteristic, that they are very hard to cultivate. Among these, most believe, are *Bacillus fusiformis* and *spirochæte dentalis* (Miller); whether any of these are pathogenic or not is a question, but one thing is certain, that they increase in great numbers in any ulcerative process in the mouth and probably do aid in the tissue destruction. Certain it is that they aid decomposition of the exudate in these ulcers, and explain much of its bad odor. It is very likely that *Bacillus fusiformis* is important in the production of Vincent's angina, but it is interesting to hear the discussion aroused in the demonstration of a slide from such a case, fairly well covered with fusiform bacilli and spirochæte. For smears from ill-kept mouths without any deep ulcers will show such remarkable pictures that the smear from the base of an ulcer must be very rich indeed in long bacilli and spirochæte before many will grant it any importance in the diagnosis.

## CHAPTER II

### THE URINE

#### GENERAL CHARACTERISTICS

**The Collection and Preservation of Urine.**—It is of the utmost importance in all chemical examinations of the urine that a complete and well-mixed twenty-four-hour specimen be obtained, so much do the various voidings of the day differ. To accomplish this most patients need to be watched, and one must rely much on the attention of nurses and orderlies.

In this clinic the day's collection begins at about 6 A.M. The patient voids at this hour, and the urine is collected from then until 6 A.M. the next day including this hour's voiding. In case we separate the urine of the day and the night, the former period extends from 6 A.M. to 9 P.M.; the remaining hours are those in which the patient is, as a rule, asleep.

It is very essential that a clean bottle be employed and some means used to prevent the very rapid bacterial action. There is no one preservative which is good in all cases, and the worker should choose his agent with reference to the use to which he expects to put the urine. For instance, for chemical work we usually use chloroform, enough so that several drops remain at the bottom. The bottle must be tightly corked or bacteria will certainly grow in the upper layers from which the chloroform is volatilizing. We prefer this, since it adds nothing to the volume and can be entirely removed. The disadvantages of it are that the formed elements are not well preserved for microscopical examination, although crystals are, and that certain chemical changes do result, whereby, for instance, a suggestive sugar-test with Fehling's can be obtained in urine thus preserved; yet even for oxybutyric acid determination the urine may be kept unchanged even for years. A few crystals of thymol are often used. A slight objection to this is that the urine will give a test similar to bile. Gum camphor is very commonly used. Formalin is of value to preserve microscopical constituents. A person must be wary in a chemical examination of such an urine, since formalin is an active reducing body, and the diagnosis of glycosuria has been made. Other workers employ a dilute chloroform water or a saturated borax solution, adding one-fifth volume to the urine. The specimens, however preserved, should be kept in an ice box.

Sometimes a twenty-four-hour specimen is not desirable. For instance, in the diagnosis of slight chronic nephritis a comparison of

the urine first voided and that voided at the end of a day's work gives valuable information, also in a suspected case of cyclic albuminuria. Again, in diabetes mellitus of a very mild degree the urine voided three or four hours after a hearty carbohydrate meal may contain sugar sufficient for a positive test, while if this voiding be diluted by mixing it with the whole twenty-four hours' amount the sugar percentage would be too small to be detected. For microscopical examination the urine should be tested as early as possible after voiding, and, if possible, without the addition of any preservative. Formalin is said to add a crystalline component to the sediment (May).

The **value** of urinary diagnosis as a routine practice cannot be too strongly emphasized. About fifteen minutes are sufficient to find out if anything unusual demands further attention. The doctor or student who employs the "X-ray test" or the "sink test" is a traitor, and should be treated as such.

The unexpected is found quite often, and the perfectly healthy appearance of the patient is no guarantee that the urine will not clear up the case. The surgeons especially need this warning. A recent case was a lesson, for one urine examination would have probably prevented an operation following which the woman went into diabetic coma and died.

**The Amount of Urine.**—The limits of the amount of urine to be considered normal vary widely, both for individuals, and depending upon this, for different countries, especially those in which the customs are fairly uniform. In general it depends on the amount of water in the food and of solids in the blood, especially salts, to be excreted, their excretion increasing the water output. The limits usually given are from 1500 to 2000 cc. That may be true for a country in which beer-drinking is very common; it is, however, too high for others, as for this, where from 900 to 1200 are more common figures. For France the figures 900 to 1500 are given (Becquerel). In women the output is slightly less than in men. The amount of urine also depends on the size of the person; in an adult it is almost directly proportional to his weight. This is not true in the case of children, who excrete relatively more than do adults; newly born infants, from 150 to 200 cc. a day, and children from three to five years of age, about 700 cc.

The amount depends chiefly on the volume of fluids consumed. The extreme physiological limits, depending chiefly upon this, are from 800 to 3000 cc. The increased output reaches its maximum in from two to three hours after drinking a large amount of water, and is over in from five to six hours. Yet, as several have shown and all experienced, the water output is perhaps the most capricious of all the urinary constituents, and the water ingested is only one factor in the question.

The functional limits of the kidney are something enormous, as is seen in diabetes mellitus, in which a practically normal kidney may eliminate 25 litres of urine, an absolutely increased amount of the normal solids, and several hundred grammes of an abnormal solid, sugar, and stand this increased work for some time without any sign of disease. Külz was able in a rabbit by intravenous injection of salt solution to increase the urine to 256 cc. per hour for nine hours, and yet the qualitative composition of the urine remained normal. Insensible and especially copious perspiration affects the amount of urine, which is therefore greater in cool weather than in hot. The latter, however, can be really no great factor, since then a person drinks more. It is also affected by the amount of fluid lost in other ways, particularly by diarrhoea and by vomiting.

Exudates (pleural or ascitic), œdema, and other abnormal accumulations of fluid in the body are excreted through the urine. This explains the polyuria in nephritis as the œdema disappears. It is beautifully seen if the person be put on constant fluid and the urine carefully measured; yet here also the excretion is not immediate, and may be distributed over so long a time that the demonstration fails.

The relative amount of urine voided during the *day and night* has not received the attention which it deserves. Quincke was first to call attention to this point. He and his students found that in liver, kidney, and heart diseases producing œdema the urine voided per hour during the night is greater in amount and contained more solids than during the day, a condition sometimes called *nycturia*. Normally the reverse is true, the kidneys seem to sleep with the rest of the body, and the amount per hour during the day is to the amount voided per hour during sleep as 100:50 to 60 or perhaps 80 to 90. The reverse is true in cases of cardiac or arterial disease and in nephritis, in which cases it would seem as if the kidney during the sleeping hours improved its opportunity to eliminate that which it could not during the day. In a well-marked case of nephritis, D:N::100:200, but in one case which we followed the ratio was even 100:544.<sup>1</sup> This does not depend, we are convinced, upon the mere position of the patient and the circulatory changes dependent upon this. This has some diagnostic importance to differentiate those cases of functional (*e.g.*, hysterical) from organic disturbances. The disturbed ratio is particularly marked in case the output be increased, as in diabetes, or by diuretics or by exercise during the day. It is not found in heart disease providing the compensation be good. Cardiac insufficiency seems the underlying cause in all cases.<sup>2</sup>

By **POLYURIA** is meant an increased output of urine, 3000 cc. being

<sup>1</sup> Johns Hopkins Hosp. Rep., vol. x. p. 323.

<sup>2</sup> See Laspeyres, Deut. Arch. f. klin. Med., August 16, 1900.

roughly considered as the upper physiological limit. If the output be below 800 cc. the term *oliguria* is used. The observation of one day is never sufficient; the increase or the diminution must extend over several consecutive days. These limits are very elastic, the controlling factor being the amount of fluids ingested, and the question always arising, is the polydipsia primary or secondary to the polyuria? For instance, in the cases of typhoid fever without any apparent renal disturbance Dr. Cole has been in the habit this year of increasing the diuresis as much as possible in hopes of increasing the elimination of the toxines, and outputs of from 6 to 14 litres a day were not rare. Here the polyuria was secondary to the consumption of larger amounts of water than the patients desired. In other cases also of typhoid fever after convalescence the output of urine is increased perhaps from the elimination of certain solids, and the increased intake of fluids is secondary to the tissue-thirst intensified by the depletion of water.

PATHOLOGICAL FACTORS INFLUENCING THE AMOUNT OF URINE are:

(1) The condition of the renal parenchyma; a bilateral diffuse lesion is usually necessary. The general law is that the more acute the nephritis the less the amount of urine, the more chronic the nephritis the greater the amount of urine excreted. In acute nephritis there may at first be anuria, or 50 to 100 cc. only, in a subacute nephritis about a normal amount, while in a chronic interstitial from 6 to even 12 litres in twenty-four hours. In the chronic cases the reason for the polyuria is uncertain. It cannot be blood-pressure alone.

(2) The velocity of the blood current through the kidney is of particular importance, the general law being that the amount of urine varies directly as the rapidity of blood-flow, not blood-pressure alone; that is, as the amount of blood passing through the kidney in a unit of time. Hence all cases of chronic passive congestion of the renal circulation due to whatever cause have a diminished output, and drugs which improve this circulation are called "diuretics." This is important in diagnosis and in prognosis.

(3) Disturbed metabolism. The output of urine depends much on the quality and the quantity of the substances excreted. The best illustration of this is diabetes mellitus, in which disease, because of the sugar elimination, even 25 litres of urine may be voided, and when, by modifying the diet, the sugar is much diminished, the water output diminishes as well. Similar may be the explanation of the so-called "epicritical polyuria." Some cases of typhoid fever, for instance when convalescence begins, void from 4 to 6 litres of urine per day; and in almost any disease causing diminished output, as the case improves the urine is much increased. This is beautifully seen in cases of nephritis, especially the chronic parenchymatous. The increased



water output following fevers, and also that of subacute nephritis, may accompany the elimination of substances which were retained during the acute periods of the illness. This polyuria indicates a favorable prognosis.

(4) Psychical disturbances and various nervous storms may be followed by polyuria, as may also angina pectoris, hysteria, and epileptic convulsions. The cause is probably a vasomotor one. The so-called "paroxysmal polyuria" is probably a functional disturbance.

(5) Another cause of periodic polyuria is the periodic hydro-nephrosis seen in movable kidney, etc.

(6) Chilling of the skin is followed by polyuria.

(7) There are certain other cases of polyuria the causes of which are unknown. The best illustration is diabetes insipidus, in which the output may be as high as twelve or more litres in a day. Meyer,<sup>3</sup> whose paper is a very interesting one, considers that in this disease the kidney is unable to secrete urine of normal concentration, and so abnormal amounts of water must be excreted in order to eliminate the normal amount of solids.

In certain diseases the sequence is perhaps the following: The renal cells, owing to disease, to circulatory disturbances, or to the excretion in abnormally large amounts of some solid, eliminate a greater percentage of the water of the blood than normal. The concentration of the plasma leads to a somatic thirst, hence the ingestion of an increased amount of fluid which is at once excreted. On the other hand, in the case of acute nephritis an oliguria may be due to the functional insufficiency of the cells to excrete water and salts.

It is often of interest to note what proportion of water intake is excreted through the kidneys. For normal persons 60 to 70 per cent. may be considered the usual limits, although the influencing factors are many. If the water consumed be very much increased, the bulk appears in the urine and the percentage rises to even 96 per cent. (a case of typhoid fever, with ingestion of 6772 cc. of fluid). In two cases of chronic interstitial nephritis the relative output through the kidneys was high even when this output was small; one case with an intake of 1960 cc., 85 per cent.; the next day, of the 2400 cc. consumed, 86 per cent. were excreted by the kidneys. In another case, of 1370 cc., 85 per cent., and of 1700 cc. 83 per cent. were thus eliminated. In chronic parenchymatous nephritis, with the patient in almost stable condition and receiving exactly the same amount of fluid each day for 26 days (6200 cc. total), the average output was 66 per cent. With ascites and other signs of renal insufficiency it will drop to 40 per cent. or lower, even in anuria to 0. The following figures from a recent case of eclampsia in the obstetrical ward will illustrate this well. The patient was not urged to drink much. On the first day after the convulsions, of 8350 cc. of water drunk, the kidneys excreted 20 per cent.; the next day, of 10,535 cc., 80 per cent.; on the fourth day, of 9400 cc., 93 per cent.; and on the fifth the 7300 cc. of urine exceeded the intake of 7100 cc. During this time there was also some diarrhœa.

ANURIA may be due to a variety of causes, which may be grouped as obstructive, reflex, renal, and prerenal. It may be simply a nervous

<sup>3</sup> Deut. Arch. f. klin. Med., 1905, Bd. lxxxii.



symptom, seen in hysteria, in which case it is followed by a polyuria. It may be due to trauma, to the occlusion of the urinary passages, as, for instance, by stones, or a stone on one side and reflex anuria on the other, or to the reflex influence of nephrectomy on one side; or to the condition of the renal epithelium as in acute nephritis, tuberculosis, cystic disease, etc. There are many so-called "pre-renal" causes of anuria. Among these are: certain fevers, as scarlet fever; certain poisons, as phosphorus, lead, turpentine, ether, and chloroform; collapse; and often, but not always, approaching death.<sup>4</sup> In cholera the anuria is attributed to inspissation of the blood. Moxon reported a case of ureteral calculus with anuria lasting fourteen days, and recovery after the passage of the stone. Adams's patient had anuria for nineteen days and yet recovered. Polk's patient lived for eleven days after his one and only kidney was removed.

**Specific Gravity.**—By specific gravity of the urine is meant its weight compared with that of an equal volume of water. The latter is usually expressed as 1000. This may be determined accurately by weighing in a pycnometer, but clinically it is determined by a form of aërometer called a urometer. These spindles are usually graduated from 1000 to 1050. It is better to use two, one graduated from 1000 to 1020, the other from 1020 to 1040. The practitioner should get good instruments, as some on the market are inaccurate, especially those designed for use with a small volume of urine. The specific gravity of the urine can be put to good use, and the best instrument is none too good. The urine glass used should be a cylinder with parallel sides, wide base, and a good spout. The fluted side of the Squibb's model is an advantage. This glass is filled about four-fifths full of urine in such a way as to avoid foam, which, if present, may be removed with a piece of filter paper. The bobbin is then dropped in. The observer now assures himself that it neither rests upon the bottom nor touches the side of the glass. If it does touch the side it will register from 1 to 2 points higher than it should. The reading is made with the eye on a level of the base of the meniscus. Two or three readings should be made, the bobbin being pushed down and allowed to come to rest each time. There is one point of considerable importance, and that is that these instruments are standardized at a certain temperature, usually at 15° C., and a difference in temperature of 3° means a difference of 1 in the fourth place of the specific gravity reading. Hence a urine which at 15° C. has a specific gravity of 1012, at 18° C. will read 1011. This is, of course, usually of slight importance with urines of ordinary concentration, yet we suspect that it explains the phenomenally low specific gravity in certain cases of diabetes insipidus and chronic interstitial

<sup>4</sup> See, also, Bevan, *Am. Surg.*, April, 1903.

nephritis. This correction is, of course, indispensable if the specific gravity is to be used in quantitative work, as, for instance, the estimation of the total solids or the amount of sugar or of albumin. It is only just to say that for the latter we think the aërometrical method at its best is hardly accurate enough, and the urine should if possible be weighed on a good chemical balance. Again, an instrument suited for salt solutions is not always accurate in a sugar or albumin solution.

The twenty-four hours' specimen should be examined; this only has very much value, for the various portions during the day and night may vary from 1002 to 1040, depending on the food, the fluid, the lungs, the skin, etc. It may be very high after severe exercise with sweating, after transudate formation, etc. Two cases recently were refused on first examination by life insurance companies because they happened to have eaten some food just before examination which for them was a diuretic, hence an abnormally low specific gravity was found—in one case as low as 1003.

The normal specific gravity is from 1015 to 1020. In the newborn, 1005 to 1007.

In case there is too small an amount to fill the tube, it may be diluted to a known volume, the formula for the correction being:  $\text{Sp. gr.} = 1000 + ab$ , in which " $b$ " = dilution, and " $a$ " = the last two figures of the specific gravity found. For instance, if the urine was diluted with just twice its volume of water, and if the reading of the diluted urine was 1006,  $\text{Sp. gr.} = 1000 + 3 \times 6 = 1018$ .

In some cases it is not the specific gravity of the twenty-four-hours' specimen which is desired. For instance, in the diagnosis of an early chronic diffuse nephritis the constantly low specific gravity of the morning urine is of value. In general, however, if one has not a total mixed specimen, the specific gravity would better not be determined. This figure is in some clinics put on the temperature chart together with the amount of urine, the reason being that neither figure means much without the other.

The specific gravity depends chiefly on the amounts of water, urea, and sodium chloride present. The water will depend on the same factors already discussed under amount. The urea explains to a certain degree the high specific gravity in fevers. The amount of salts is increased by foods, by the medicines taken, and by the absorption of transudates. While in general the specific gravity will vary inversely as the amount, this is not strictly true, since the output of solids is always increased by an increased output of fluids. A noted exception is that of diabetes mellitus, in which with increased amount is also an increased specific gravity, from 1025 to 1040; and in nephritis with renal insufficiency, in which case with oliguria the solid

output is diminished. In nephritis a low specific gravity is rather suggestive of an impending uræmia. It is also seen, however, in cases of malnutrition in which the metabolic processes are at low ebb, as for instance in a patient of Chabrié, a girl of twenty years of age, whose output on one day was 750 c.c. with a specific gravity of 1008. In diabetes insipidus the specific gravity is very low (some very low figures are probably explained by a failure to make a correction for temperature). After operations, in rheumatism, and in rickets, there is a rather high specific gravity. Ether anæsthesia diminishes the specific gravity, the amount remaining about normal (Brown).

To determine the amount of solids in the urine an approximate estimation may be made by the use of Häser's coefficient, 2.33. The last two figures of the specific gravity multiplied by this empirical coefficient will give a fairly accurate estimation of the number of grammes per litre of solids excreted.

Others disagree concerning this coefficient. Neubauer gives 2.328; Donze<sup>1</sup> states that the coefficient should be slightly lower for dilute than for more concentrated urines, varying from 1.850 to 2.440, with an average of 2.210.

The simple determination of the specific gravity is useful in quantitative work; *e.g.*, sugar, albumin, etc. If used for this purpose, however, the observations should be present on several successive days, since the physiological variations of renal function are marked.

**Color.**—The color of normal urine is usually a shade of yellow. This varies with the dilution of the urine, and hence directly with its specific gravity, a dilute urine being of pale, and a scanty urine of dark color. Exceptions to this are: diabetes mellitus, in which case the urine is very pale and yet increased in amount and of a high specific gravity, a point which will sometimes suggest the diagnosis; in some anæmias, especially chlorosis, in which cases the urine is pale from lack of pigment, since hæmoglobin is the chief source of the urinary pigments, but in those anæmias in which there is destruction of the red corpuscles, as in pernicious anæmia, the urine is highly colored. As a rule, acid urine is more highly colored than is alkaline. In uræmia the urine often is pale, a fact which is responsible for the old theory that a retained pigment is cause of the condition. In certain grave infections which seem to destroy the bile-producing function of the liver the urine is said to be without any pigment. A febrile urine is dark, since it is concentrated, and also because it contains considerable uroerythrin and other pigments. This color is manifest after the urine is exposed to the air. The contrast in color between the day and night urine is often striking, the day urine being of a golden-yellow, the night of a pale green color.

<sup>1</sup> Compt.-rend. Soc. de Biol., 1903, 155, 537.

This is due partly to the amount of pigment excreted, partly to the effect of sunlight on the specimen collected during the day.

A color scale is very convenient to use (such may be found in Purdy's "Analysis of the Urine," and Neubauer and Vogel's chart is published large for the urine examination-room by Kreidel, of Wiesbaden), since a variety of terms is used in describing the same color (yellow, light yellow, amber, straw, etc.) With this scale should be compared urine in vessels of a certain depth, and against a white background.

The pigments normally present in the urine are:

**UROCHROME** which is the one chiefly responsible for the normal yellow color. This is the predominant pigment of the urine, giving colors varying from yellow, orange, to brown, according to the amount present. It has not yet been isolated, its empirical formula is not yet known, and there may be several pigments included under this name. It has no absorption spectrum, no fluorescence. There is evidence that it is derived from urobilin.

**HÆMATOPORPHYRIN** is in small amounts normal in the urine (see pages 104 and 252).

**UROERYTHRIN** is normal in certain cases. It explains the salmon-red color of the urate sediments. It is increased by a rich meat diet, profuse sweating, alcoholic drinks, violent exercise, and by certain digestive disturbances; also in fever, circulatory disturbances of the liver, and rheumatism. It may be demonstrated by shaking the urine out gently with amyl alcohol, which will take an orange color and give the characteristic spectrum. This pigment bleaches in a characteristic manner on exposure to light. With concentrated sulphuric acid its solutions are carmine-red, which on the addition of an alkali changes from purple, to blue, to green.

**UROBILIN** is a constituent of the normal urine, in amounts varying from 30 to 120 mg. per day. Urobilin itself is not present in perfectly fresh urine, but its chromogen, urobilinogen, is, and this on exposure to sunlight yields urobilin. In the following lines we shall include under the term "urobilin" its chromogen or chromogens.

Whether urobilin is a single pigment or a group of pigments is a doubtful question. It has been impossible to isolate it without some decomposition, and all efforts to remove impurities from the substance isolated have thus far failed.

The origin of the urobilin in the urine is still undetermined, but evidence favors most the theory that it originates in the intestines and the liver. There is no constant relation between urobilinuria and urobilinæmia, or between bilirubinæmia and urobilinuria. The attractive theory of Gilbert and Herscher and others,<sup>6</sup> that the kidneys

<sup>6</sup> Compt.-rend. Soc. de Biol., 54, p. 795.

transform the bilirubin in the blood into the more diffusible pigment, urobilin, has not received recent confirmation. Conner and Roper\* found that as a general rule bilirubinæmia and urobilinuria go hand in hand and usually bear a rough quantitative relation to each other, though the exceptions to this rule are so frequent and important that it can hardly be said to furnish much support to the theory that the urobilin of the urine originates in the kidneys. Certainly a great deal of urobilin is formed in the intestine (enterogenous formation), and is a product of the reducing action of certain bacteria on bile pigment. It seems to be identical with stercobilin.

The presence of abnormal amounts of urobilin in the urine is considered by some evidence of hepatic insufficiency, indicating a diffuse lesion.

A certain amount of urobilin is said to be formed in areas of blood extravasation in the blood tissues (histogenous formation), and, indeed, as the result of blood destruction from any cause (hæmatogenous formation), as after toxic doses of blood poisons, such as antifebrin and antipyrin. Meinel<sup>7</sup> found that a certain amount is formed in the stomach in some cases of hyperacidity.

The urobilin of the urine is increased also in fevers, chronic passive congestion, lead poisoning, atrophic cirrhosis of the liver, etc. It is increased before and after a period of obstructive jaundice. There is a life-long increase of it in patients with chronic family jaundice (Tileston and Griffin).

When there is a marked urobilinæmia there may be a definite *urobilin jaundice*.

Urobilin does not give the Gmelin test. It does give a test similar to the biuret. If to the urine made strongly alkaline with ammonia and filtered be added a 1 per cent. alcoholic solution of zinc chloride, there will be seen a beautiful green fluorescence, and the absorption bands of alkaline urobilin may be found. This spectrum is characteristic. That of acid urobilin may be determined in a urine directly if a few drops of a mineral acid be added, but it is better to shake out with amyl alcohol and examine the extract. Or to the urine may be added an equal amount of 10 per cent. ZnAc in absolute alcohol, and the mixture filtered.<sup>10</sup> This test is given even in the presence of considerable bilirubin. The fluorescence is best seen with a convex lens, which gives a luminous green circle.

For quantitative work, Hoppe-Seyler's method is recommended. One hundred cc. of urine are acidulated with sulphuric acid, saturated with ammonium sulphate,

\* Arch. Int. Med., Jan., 1909, vol. ii, p. 532.

<sup>7</sup> Centralbl. f. inn. Med., 1903, vol. xxiv, p. 321.

<sup>10</sup> Schlesinger, Deutsch. med. Wochenschr., 1903, No. 32, p. 561.

and allowed to stand for some time; then filtered, and the precipitate washed with saturated ammonium sulphate. The precipitate is then pressed out between blotting-paper, extracted with equal parts of alcohol and chloroform repeatedly. The extract is then filtered into a separating funnel, and to the filtrate is added two volumes of water and then chloroform until the chloroform settles out well in a clear layer. The chloroform solution is evaporated on a water-bath and the residue dried at 100° C. It is then extracted with ether, the ether extract filtered off, the residue dissolved on the paper in alcohol, again brought into the weighed beaker, evaporated, dried, and weighed.

The spectrophotometric method of Friedrich Müller may be used.

Among other chromogens in the urine are indoxyl-sulphuric acid, indoxyl-glycuronic acid, perhaps skatoxyl-sulphuric and skatoxyl-glycuronic acid. Pathologically, among the pigments present may be hæmoglobin, methæmoglobin, hæmatin, bile pigments, melanin, and others; from drugs, chrysophanic acid *et al.*; from the foods, the pigments of various berries, cherries, etc.

**BLOOD.**—The color of the urine when blood is present depends upon the amount and form of the blood pigment, hæmoglobin giving in general a reddish tint, and methæmoglobin a brownish one. The urine may therefore grossly be of a reddish-brown, brown, almost black, or greenish-black, as in the black-water fever of hæmoglobinuria. When little is present it often has a characteristic smoky tint of methæmoglobin, which should always suggest blood. The urine is cloudy because of the large number of corpuscles and other organized elements of sediment usually present. In the heavy sediment are masses of amorphous hæmoglobin.

**HÆMATOPORPHYRIN.**—This is present in large amounts after the long use of trional, sulphonal, tetronal; also in cases of typhoid fever and other diseases. Thick layers of the urine have a dark or a blackish color; thin layers a yellowish-red or violet. The black color Garrod thinks due only partly to this pigment, and more to an unstable purple one.

**BILE.**—When the patient is jaundiced the urine usually contains bile, but in cases of very mild jaundice urobilin alone may be present. If bilirubin and biliverdin are present, the color of the urine will often be dark yellow, brown, green, or even greenish-black or quite black if considerable biliverdin is present together with bilirubin and other bile pigments, especially in long-standing cases (Garrod). If it stands a long time in the cold there may be a sediment of bilirubin in needle crystals, especially if the urine is very acid. It is often possible to detect the presence of bile in small amounts by producing a foam by shaking the urine. This foam, always white in other urines no matter how dark they may be, is stained yellow by bile; it is also yellow in case very much urobilin is present.

**MELANIN.**—This rare pigment is present in cases of melanotic tumors which have invaded the viscera. Garrod finds the amount

of melanin to depend upon the involvement of the liver especially. The urine is usually of a perfectly normal color when voided, since the pigment is present as a chromogen, melanogen, which later splits giving melanin. But it may be black when voided. This transformation may be hastened by the addition of nitric acid or other oxidizing bodies to the urine. It begins at the top and extends downward forming sometimes very strikingly a sharply defined layer above the colorless urine. Ferric chloride causes immediate blackening and a gray precipitate soluble in excess. Unless this reaction is positive, melanin cannot be assumed present. This is the most delicate and reliable test. (v. Jaksch.)

HOMOGENTISINIC ACID, the chief coloring body of alkaptonuria, gives the urine a brownish-black color and a syrupy consistency after standing or after the addition of an alkali (see page 213).

The urine is sometimes very dark in peritonitis, gangrene, and other conditions with the formation of aromatic products of decomposition, the ethereal sulphates of indoxyl, etc. In these cases the blue color sometimes seen is not indigo, but a higher oxidation product of indol. Such urines blacken on the addition of nitric acid, if warmed, but not if cold. They do not blacken with ferric chloride, and do not reduce copper solutions. In one striking case in our wards the fresh urine of a woman who had been markedly constipated was of a very dark greenish-black color, but after the bowels had moved well the next voiding was of practically normal color. Some indican was present, but not nearly enough to explain the color.

In some cases the urine is very dark on voiding; in others after long standing. This may be due to *pyrocatechin*,  $C_6H_4(OH)_2(1, 2)$ , which in watery alkaline solution is oxidized by the air and becomes a greenish-brown and finally a black color. The urine containing it becomes therefore dark, reduces alkaline copper sulphate in warm solution, but not bismuth. Another view (Baumann) is that pyrocatechin is derived from the vegetables of the food.

To isolate, the urine is concentrated, filtered, a little sulphuric acid added, and then boiled to drive off the phenol. It is then shaken out repeatedly with ether; the ether is distilled off, the residue neutralized with barium carbonate, and shaken out again with ether. The ether is then evaporated off and the pyrocatechin allowed to crystallize out.

*Hydrochinon*,  $C_6H_4(OH)_2(1, 4)$ , occurs after the use of phenol. Its decomposition product gives a dark color to the urine and reduces copper easily.

Urine containing the alkapton bodies and indican is clear on voiding, but soon becomes dark. In the latter case the blue of the indigo may not be pronounced, as it is modified by the yellow of the urine. The scum, however, may be blue. Sahli mentions the case of a boy



in which the urine when voided was of a green-grass color due to the combination of the indigo with the yellow of the urine.

*Ochronosis* is a rare disease with blackening of the cartilages. The urine of these patients turns black on standing. Osler reported two cases of *ochronosis* with *alkaptonuria*, but in other cases it is said that the black color of the urine was not due to *alkaptonuria*.

In certain cases the urine on voiding is black. In others it is first colorless but soon turns black. The pigments in these cases have not been determined, but all the above mentioned causes it is said are excluded.

Garrod<sup>11</sup> classifies the *black urines* as, those due to long-standing jaundice; certain cases of *hæmaturia*, *hæmoglobinuria*; *melanotic sarcoma*; *alkaptonuria*, *ochronosis*; great abundance of *indoxyl-sulphate*; certain cases of *tuberculosis* after standing for some time, a month even (the cause not known); perhaps *phenol derivatives*, certain drugs as *phenol*; and rare cases due to an unknown pigment. Those truly black are only *melaturia*, and *alkaptonuria* on standing.

In *chyluria* the urine is of milky appearance.

COLORS DUE TO MEDICINES.—The list of medicines which may affect the color of the urine is too long to tabulate. In general, it may be said that in case the urine presents any unusual color, inquiry should always be made concerning the previous medication. Among these drugs particularly are *carbolic acid*, whether applied internally or externally, *tar preparations*, *resorcin*, *naphthol*, *salol*, and many aromatic bodies. The color in these cases often appears only after long standing, and especially when the urine is alkaline, and when *hydrochinon* and *pyrocatechin* are formed. *Methylene blue*, even in small amounts, 0.1 gm., will color the urine for several days. Hence the result was startling in cases of *malaria* treated with large doses of this drug. In one hour after the dose the urine has a greenish color, later a deeper green, then a blue, which may last three to four days. The color may be intermittent, present only in the first morning voiding. It may be intensified or produced by boiling the acid urine, adding *acetic acid* if necessary, since the pigment is partly reduced in the body to a colorless form. Weber<sup>12</sup> thinks *methylene blue* explains practically all the blue and green urines, and doubts cases ascribed to *indigo blue*. He emphasizes the common use of this dye to color candies and food-stuffs.

Some colors are of clinical importance only in case of a drug applied externally and hence in uncontrollable doses. What the factor is which changes the color of the urine cannot always be determined; for instance, after a small dose of *salol* the urine may be of a very

<sup>11</sup> The Practitioner, 1904, vol. lxxii. p. 383.

<sup>12</sup> Lancet, September 21, 1901.



dark color, while after much larger doses there will be no change. Whether this is due to the acidity or to the time of exposure to the air cannot be said.

After drugs containing *chrysophanic acid*, as, for instance, chrysarobin, rhubarb, santonin, senna, and others, the urine is of a yellow tint when acid and red when alkaline. The pigment of many vegetables will change the color of the urine. Among these may be mentioned turnips, whortleberries, blackberries, and others.

**Odor.**—The odor of the normal fresh urine is not unpleasant. The so-called urinary odor is due to the ammoniacal decomposition by the bacteria. In a decomposing albuminous urine the odor is especially disagreeable, and a diagnosis of albuminuria may be made from that alone. There is said to be an intolerable odor in cases of cancer of the bladder and deep inflammatory disease of the urinary tract. Chabrié believes in a characteristic odor in certain cases of abnormal metabolism with incomplete combustion, such as is present in diabetics and oxalurics. We may even suppose that he thinks that one of the great masters of French medicine could diagnose insanity from the odor of the urine alone. There is said to be a special odor in chyluria and even in slight hæmaturia. Other cases have a remarkable absence of odor. It should always be remembered, however, that the bottle in which the patient brings the specimen may explain the odor. We have noticed a strong odor of  $H_2S$  in certain nephritics, even when the urine was quite fresh.

Certain substances are excreted as such in the urine. Among such are valerian, asafetida, coffee, and various foods. Others build odorous bodies. Among these are the balsams, copaiba, cubebs, etc. After the administration of turpentine the odor of the urine is that of violets. After eating asparagus there is a characteristic odor attributed to methyl-mercaptan.

**General Appearance.**—When fresh the urine is clear. If there is then any distinct cloudiness, it is due to an abundant organized sediment or to a precipitation of phosphate seen in the so-called phosphaturia and in ammoniacal cystitis. Very soon a faint nubecula appears in the upper layers of a clear urine, which consists of mucous strands enclosing a few cells. After standing, the urine will become cloudy, either from a urate sediment, which before settling may give a uniform milky appearance, or particularly during the summer months to the rapid growth of bacteria and the precipitation of the phosphates in the alkaline urine.

**Reaction.**—Concerning the reaction of the urine there has been much work done in regard to the value of which much difference of opinion exists. All admit that its determination would be valuable could satisfactory methods be found.

Until recently by "degree of acidity" was understood the amount of hydrogen which could be replaced by the metal of an alkaline solution (NaOH), regardless whether these hydrogen ions were already dissociated or could be substituted by the alkali. Now is meant the absolute number of dissociated H-ions per one litre of urine. The latter contribution of the physical chemists is interesting, indeed, but of little value to the clinician. Judged by this, urine is only about thirty times as acid as distilled water, and only about one ten-thousandth as acid as titration would indicate, and the difficulties of its determination rule this out from clinical methods. The titration method alone is possible for general use, and the question arises if its results are of any real value or simply of an empirical arbitrary value. Höber<sup>13</sup> claims, as the result of parallel estimations, that these two "acidities" vary sometimes, often perhaps, in a very independent manner, hence variations in each would have different values, and neither method would be able to replace the other. The question, therefore, is, Does the titration method give results valuable enough to repay the time, or are the results worse than useless since misleading? The difficulties are that the acidity of the urine in the common sense of the term depends upon a considerable number of chemical substances, for the most part acid salts, and hence the question of color indicator is a very serious one, since the points indicated by the various ones as the neutral point differ and none is by any means the theoretical one. Phenolphthalein is the one usually used. This has as practical advantages the sharpness of its end reaction and the fact that of the indicators it is itself the weakest acid. But it is a poor indicator in the presence of ammonium salts, perhaps the worst. Whatever results are obtained with it must be given not an absolute but an empirical value. Yet the opinion of those working in this line is that the results with it are comparable.

The reaction of the twenty-four-hour amount of well-preserved urine is, in the case of man, always faintly acid to litmus, a degree corresponding to about 1.15 to 2.3 grammes of HCl for twenty-four hours. This acidity depends chiefly upon the diet, and is greater the more the proteid oxidized. The urine of herbivorous animals is alkaline, since the organic acids of their food are oxidized to alkaline carbonates, yet if starved, acid, since then their tissue proteid is their diet. A man on a vegetable diet will have a less acid, or amphoteric urine perhaps, from this increased ingestion of alkali-forming foods. In no case is there free acid in the urine, the acidity being due to acid salts, and particularly diacid sodium phosphate. There are many other acids produced in the oxidation of proteids themselves neutral. Among these are sulphuric, phosphoric, uric, hippuric, oxalic, and the

<sup>13</sup> Hofmeister's Beitr., 1903, vol. iii: p. 525.

oxyaromatic acids. Just what part these play, however, cannot be decided, but certainly uric acid is no factor, since its solution is neutral to litmus.

A constant acidity is found only after and during starvation.

Variations in the reaction are due to the diet, as mentioned above. The acidity is highest in the morning before breakfast and lower a few hours after each meal, and especially in the forenoon, due to the secretion of hydrochloric acid of the gastric juice. For a short time, from two to four hours, after a meal the urine may be alkaline when freshly voided and turbid with sediment of the phosphates of the alkaline earths. This condition is known as "phosphaturia." Normally this diminished acidity of the urine, known as the "alkaline tide," disappears after a meal, since then the hydrochloric acid is reabsorbed.

PHOSPHATURIA is the term given to a symptom-complex with a heavy precipitate of the earthy phosphates in the freshly voided urine, yet without the formed elements which would indicate a lesion of the tract. It was supposed to be due to an increased output of phosphoric acid. Chemically, however, there is no such increase, but often a decrease, and so the name "alkalinuria" is more suitable. Phosphaturia occurs when the diet raises the alkalinity of the blood, as will a vegetable one; in gastric diseases with considerable loss of hydrochloric acid to the body through hypersecretion with motor insufficiency and vomiting or lavage, perhaps diarrhoea also; and especially as a symptom of neurasthenia (Peyer) without any of the above-mentioned causes. In such a case during the periods of neurasthenia has been found a diminution in the phosphoric acid to about half, but an increased calcium output. The nitrogen was also decreased. It seems to be the excess of calcium relative to the phosphoric acid which leads to the precipitation. In Soetbeer and Krieger's case the phosphoric acid was practically normal, the calcium increased even to 0.7 gm. a day (normal 0.2) and  $\text{Ca}:\text{P}_2\text{O}_5::1:1.5$  to  $2$  (normally  $1:12$ ). In certain cases<sup>14</sup> there seem to be during the period of phosphaturia symptoms referable to this abnormal metabolism and which disappear with it. They seem, however, to be due to changes in calcium metabolism rather than to those in that of phosphoric acid. In one case the calcium was increased over three times, perhaps the result of catarrh of the colon. It occurs also in persons after sexual excesses, and in the depression following psychical exaltation, in which cases the cause is not known, but a nervous control is suspected. Freudenberg<sup>15</sup> carries this idea to extremes, separating

<sup>14</sup> Soetbeer and Krieger, *Deut. Arch. f. klin. Med.*, 1902, vol. lxxii. p. 553; Patek, *M. J.*, vol. xxx.

<sup>15</sup> *Deutsch. med. Wochenschr.*, September 17, 1903.

phosphaturia, latent phosphaturia (in which the precipitate appears on heating the fresh urine), and ammonuria (tested by moist litmus over the mouth of a tube of heated urine), three grades, he thinks, of the same abnormality, which he found in sexual neurasthenics especially, but not in patients with hysteria. It is often found among mental cases (Heinicke). Some few cases with general symptoms have really increased phosphoric acid as their only objective sign; later, perhaps, polyuria or glycosuria. Senator suggests that some cases of diabetes insipidus with rather high specific gravity may belong here.

The reaction of the urine can be much modified, even made alkaline, by drugs, particularly by alkaline salts in large doses. Milk of lime will give an alkaline urine due to the presence of ammonium carbamate (Abel). While a transudate is quickly absorbed the urine may become alkaline; also after hemorrhage into the intestine, in which case the blood salts are absorbed. It is alkaline in certain cases of pneumonia, typhoid fever, and diseases of the central nervous system. We have noted a marked alkalinity in certain cases of nephritis, particularly of the severe chronic parenchymatous form with much œdema, which renders the examination of casts difficult. The urine is also alkaline when there are alkaline secretions and exudates of the urinary tract, as in cases of cystitis or urethritis; and lastly in alkaline fermentation in the bladder.

The alkalinity is, of course, usually due to the changes occurring after the urine is voided. Bacteria begin at once to break the urea up into ammonium carbamate and carbonate.

It is of importance to determine whether the alkalinity is due to a fixed alkali or to ammonia. If to the latter, it is always the result of bacterial fermentation. Which it is may be determined by wetting red litmus paper in the urine and then drying it; if the alkalinity is due to ammonia, the red color will return when the paper dries. Or, moist litmus paper is hung in the mouth of the bottle. It will, if much ammonia be present, turn blue. But even normal urine contains a certain amount of ammonia, and hence the paper if left long enough will usually turn slightly blue.

The acidity of the urine can with difficulty be increased, and not beyond a certain point. This occurs with increased proteid metabolism. Cases of hyperacidity, even two to five times normal (phenolphthalein as indicator), and accompanied by symptoms of cystitis, pain especially in the trigonal region, but without demonstrable lesions or assignable cause, are reported by Brown<sup>18</sup> in cases of girls and young women of distinctly neurotic temperament. He suggests that it is a neurosis of urinary secretion. The urine is very acid in diabetes mellitus if it contains considerable oxybutyric and diacetic acids. The

<sup>18</sup> Phila. Med. Jour., March 2, 1901.

question of the reaction in the so-called "uric acid diathesis" is not yet decided. The reason that it is so difficult to increase the acidity in the case of man is that the body will protect itself against an acid intoxication by an increased excretion of ammonia, thus protecting its native mineral alkaline store from depletion. This ability is present in the herbivora to a much less extent, and hence they are more easily poisoned by acids than is man.

The effect of muscular work on the urine reaction is still doubtful.

As the urine decomposes, in some cases in from six to twelve hours' standing, it becomes more acid, the so-called "acid fermentation." The reason of this is uncertain. It is inconstant and is always soon succeeded by an alkaline decomposition. Hammarsten considers it due to the reaction between the biurates and  $\text{MH}_2\text{PO}_4$ .

**DETERMINATION OF THE TOTAL ACIDITY OF THE URINE.**—Naegeli<sup>17</sup> advised to add the N/10 NaOH directly to 10 cc. of urine, phenolphthalein used as indicator. The error is at least 4 to 8 per cent.

(For the Freund method, see page 137.)

FOLIN<sup>18</sup> uses potassium oxalate in excess to rule out the error from ammonium salts and calcium phosphate. His method is as follows:

Twenty-five cubic centimetres of urine are measured by a pipette into a 200 cc. Erlenmeyer flask, one or two drops of 0.5 per cent. phenolphthalein solution added, and 15 to 20 gms. of potassium oxalate. The flask is shaken well for one minute, then at once titrated with N/10 NaOH, shaking all the time. The alkali is added until a faint yet distinct coloration is produced.

**THE MINERAL ACIDITY OF THE URINE—FOLIN'S METHOD.**—From 0.3 to 0.6 gm. of pure, dry, granular potassium carbonate is accurately weighed (within an accuracy of 0.2 mg.) into a platinum dish, and 25 cc. of urine are measured into it. (If the urine contains much albumin this should be removed by acidifying with pure acetic acid, boiling, and filtering. A trace of albumin contains too little sulphur to affect the results appreciably.) The resulting alkaline solution is evaporated on the sandbath or electric oven to dryness, and when perfectly dry the contents of the dish are burned at just below red-heat (that is, the dish should never be more than faintly red-hot) over a so-called "radial burner" giving a flame wide enough to heat the entire bottom of the platinum dish. One must be sure the gas used does not contain sulphur. If there is any doubt on this point (which is tested by burning some of the pure potassium carbonate in the platinum dish and testing the contents for sulphates) an alcohol flame may be used. If the entire bottom of the platinum dish is not evenly heated the cyanogen derivatives of urea, which resemble mineral matter, will melt, flow to the cooler portions, and escape decomposition.

The burning should continue for about an hour after all ammoniacal fumes have ceased to come off. Then the flame is removed. It makes little difference if the ash is not perfectly white. Just 10 cc. of hydrogen peroxide water are next added, the dish covered with a watch glass, and gently warmed until the peroxide is decomposed. The watch glass is then removed and the sputterings rinsed into the dish by means of a little water. The contents of the dish are again evaporated to perfect dryness, and are again heated over the radial burner as before for about an hour. The hydrogen peroxide is used to oxidize the thiocyanates and any small amount of sulphides which may have formed during the burning. Even with these precautions the complete combustion of the urine is very difficult.

<sup>17</sup> Zeitschr. f. physiol. Chem., 1900, xxx. 313.

<sup>18</sup> Am. Jour. Physiol., 1903, ix. 265.

The residue is now dissolved in water with the help of an excess of N/10 HCl (75 or 100 cc., depending on how much carbonate was used), and is rinsed into an Erlenmeyer flask, boiled to drive off the carbonic acid, and cooled. The excess of acid is then titrated with N/10 NaOH in the presence of a small amount of potassium oxalate (to precipitate the calcium) and two drops of a one half per cent. solution of phenolphthalein.

Since the amount of alkali and of acid added to the urine are known, the final titration gives the data for calculating the apparent excess of mineral acids or alkalies originally present in the urine. Before the final result is obtained certain other factors must, however, be taken into account. One must determine: (1) the alkaline strength of the potassium carbonate; (2) the acidity of the hydrogen peroxide; (3) the  $\text{SO}_3$  content of the hydrogen peroxide; (4) the preformed ammonia in the urine; (5) the inorganic  $\text{SO}_3$  of the urine; and, finally, (6) the total  $\text{SO}_3$  found in the titrated solution of the urine residue.

The potassium carbonate and hydrogen peroxide will keep for months in well-stoppered glass bottles, so the first three determinations need be made but once (for any given sample of carbonate and peroxide).

To calculate the result, one subtracts from the apparent excess of acidity found on titrating the burned urine residue the sum of the preformed ammonia, the acidity of the hydrogen peroxide and the acidity due to the organic  $\text{SO}_3$  of the urine, all in terms of tenth normal acid.

The acidity (in cubic centimetres of tenth normal acid) of the organic  $\text{SO}_3$  is obtained by subtracting the sum of the  $\text{SO}_3$  of the hydrogen peroxide and the inorganic  $\text{SO}_3$  of the urine from the total  $\text{SO}_3$  of the urine residue, and dividing the amount thus obtained in milligrammes by eight. (Eight grammes of the organic sulphur, neutral and etherial, are taken to represent 1 cc. of N/10 acid.)

To illustrate: 25 cc. of urine were burned with 0.5287 gm. of potassium carbonate (7.76 mg. of which contained 1 cc. N/10 alkali). The burned residue was boiled with 75 cc. of tenth normal HCl and the titration required 1 cc. tenth normal NaOH. An ammonia determination gave 5.2 cc. N/10  $\text{NH}_3$  in 25 cc. of urine. The total  $\text{SO}_3 = 59.9$  mg.; the inorganic  $\text{SO}_3 = 42.8$  mg. (10 cc. of the hydrogen peroxide used contained 8.8 mg.  $\text{SO}_3$  and 0.5 cc. N/10 acid.)

0.5287 gm. $\text{K}_2\text{CO}_3$	: 68.1	cc. N/10 NaOH
NaOH added	: 19.	" " "
Total alkalinity	: 87.1	" " "
HCl. added	: 75.	" " "
Apparent acidity of urine	12.1	" " HCl
Ammonia in 25 cc. urine	: 5.2	" " "
Acidity of $\text{H}_2\text{O}_2$	: 0.5	" " "
Acidity of organic $\text{SO}_3 = \frac{59.9 - (42.8 + 8.8)}{8}$	= 1.	" " "
	6.7	
Mineral acidity in 25 cc. = 12.1 - 6.7	= 5.4	cc. N/10 HCl.

THE ORGANIC ACIDITY IN URINE.—By subtracting the mineral acidity from the total acidity one obtains the "organic acidity," or rather the total equivalence of organic acid whether free or combined. In cases of acid intoxication, as in diabetes, the mineral acidity may turn out to be an alkalinity and all the acidity be organic. In the latter case the mineral alkalinity is added to the total acidity to get the organic acidity.

## THE NITROGENOUS BODIES.

**The Nitrogen Output.**—The total nitrogen of the urine is the best index of proteid metabolism. It is fortunate that for this, our stand-by in metabolism work, we have a satisfactory method of determination. The same cannot be said, however, of the several nitrogenous bodies.

Folin,<sup>19</sup> from his careful study of the urine of normal men, determined that the amount of nitrogen and its distribution was, if the diet be nitrogen-rich, that given in Table I. The figures he obtained in one case on a very low nitrogen intake are given in Table II.

	Table I	Table II
Total nitrogen .....	14.8-18.2 gm.	4.8- 8.0 gm.
Urea-nitrogen .....	86.3-89.4%	62.0-80.4%
Ammonia-nitrogen .....	3.3- 5.1%	4.2-11.7%
Creatinin-nitrogen .....	3.2- 4.5%	5.5-11.1%
Uric acid-nitrogen .....	0.5- 1.0%	1.2- 2.4%
Undetermined nitrogen ....	2.7- 5.3%	4.8- 14.6%

The figures found in most text-books for total nitrogen in the urine of the normal adult on mixed diet are from 10 to 16 gms. per day. The distributions of this nitrogen in the urine of adults and infants are, according to Hammarsten: \*

Urea .....	84 -91	73 -76
NH <sub>3</sub> .....	2 - 5	7.8- 9.6
Uric acid .....	1 - 3	3 - 8.5
Extractives .....	7 -12	7.3-14.7

The sum of the nitrogens of urea and ammonia bears a very constant relation to total N (91 to 93 per cent.), a much more constant one than does either alone.<sup>19</sup>

The most of the work done on the nitrogen of the urine is valueless since due attention was not paid to the total nitrogen of the food, the character of the food (its acid- or alkaline-producing qualities), and the age, nutritional condition and previous diet of the patient. Again the periods of observation should be at least seven days long, the diet during this time should be constant, and the daily amount of water consumed should also be constant. The patient should exercise a fairly constant amount each day. But, even when all these points are carefully watched, marked variations in the nitrogen retention and elimination will be observed.

By "nitrogen balance" is meant the relation of the nitrogen intake to the nitrogen output. The difference between these two figures is usually called the "nitrogen lost" and the "nitrogen retained." When the output is just equal to the intake the person is said to be in "nitrogenous equilibrium."

<sup>19</sup> Folin, *Am. Jour. Insan.*, 1905.

\* *Lehrb. d. phys. chem.*, 1899, p. 421.



In general the total nitrogen is increased as a result of increased proteid metabolism, a heavy proteid meal, or anything increasing body proteid catabolism. Less is excreted on a diet rich in carbohydrates than even while fasting, since in the latter case the body lives on its tissue proteid. The output reaches its maximum a few hours after a heavy proteid meal. The evidence given that exercise increases the output is in part that the amount excreted during the day is to the amount excreted at night as 3 : 2. We hardly think that this alone is sufficient evidence, for it has been only too well shown by studies of the day and night urine that normally the kidneys can rest at night as well as the rest of the body. Hot baths increase the nitrogen output.

With increase of water excretion that of nitrogen also is increased. This latter point is important, for even when the diet is fairly constant, if by any reason the amount of urine be increased, the nitrogen also will rise. One explanation given is that the renal cells are always stored with a certain amount of nitrogenous waste which the water constantly removes; others say that the many tissue ferments, following the general law of ferments, act better in dilute solution.

Pathologically nitrogen is increased: in fever, owing not to the temperature *per se*, but more likely to the effect upon metabolism of the toxins causing the fever,—excepting acute nephritis causing dropsy and diseases with diarrhoea or with large exudates: in cachexia, for in these cases there is a rapid breaking down of tissue proteid; in diabetes, since the most of these patients are on a proteid-rich diet, and the severe cases even if on a mixed diet burn only the protein of their food and tissues; after various poisons, as arsenic, antimony, phosphorus, and other protoplasm poisons; and anything diminishing the oxygen intake, as prolonged dyspnoea, hemorrhage, carbon monoxide poisoning, etc. During the resolution of a pneumonic exudate its digestion and excretion can be well followed by the nitrogen of the urine, and the amount of lung cleared estimated. In a case of Müller's the excess of nitrogen output during the resolution was 28 gms., which represented 800 gms. of exudate. The continued large output in cases of delayed resolution would indicate a "chronic pneumonia," rather than a failure to resolve. Cook<sup>20</sup> reported a few such cases, in one of which, with one lung involved, the nitrogen output would represent the exudate of four solid lungs.

In much of the work on metabolism the urea, not nitrogen, has been followed, and by methods which determine really more the total nitrogen, as, for instance, Liebig's, or those of Hüfner, *et al.*, which are quite faulty.

By combining the findings of these two lines of work, to the above may be added that in general anything increasing proteid

<sup>20</sup> Johns Hopkins Hosp. Bull., December, 1902, p. 307.



catabolism increases the nitrogen. Again, anything increasing the water output will increase the nitrogen, as, for instance, in diabetes insipidus (in which disease 130 gms. of urea have been reported), and in cases of chronic nephritis with polyuria. Again, the nitrogen is increased when exudates or transudates are absorbed.

Retention of nitrogen occurs in a person gaining weight, in myxœdema, and during the convalescence of fevers. In one case of convalescent typhoid (Lüthje) the record was reached, in twenty-six days the person retaining 121.38 gms. of N, which would represent 758.6 gms. of albumin or 3568.6 gms. of muscle. This person gained 6490 gms. in weight. This retention of nitrogen is well seen in the last stage of pregnancy, and is followed by a diuresis and increased nitrogen output, which begins about the second day of the puerperium.<sup>21</sup>

The normal daily output of nitrogen is usually stated as from 10 to 16 gms., because this is the average output of most healthy persons. But this amount is considered by some as evidence of over-eating, since men can gain weight on a diet which yields a daily output of but 5 or 6 gms. of nitrogen. Taylor's\* most careful work, continued over long periods of time, on the daily excretion of the nitrogen of normal men, shows how common are wide variations from those limits which have been considered normal.

The output of nitrogen is diminished physiologically by a "poor" diet, by reduced output of water, after profuse sweating, in pregnancy, and after small doses of quinine. The output is diminished pathologically by all conditions hindering absorption of proteins from the intestine; by all conditions which reduce the oxidization processes in the body, as in cases of cachexia and after severe fevers; by all conditions with large exudate and transudate formations, as dropsy; and by renal conditions, both organic and functional, which hinder excretion. A reduction in the amount of nitrogen excreted is sometimes an early sign of uræmia.

**Estimation of Nitrogen.**—The Kjeldahl method is quite uniformly used. Of this there are several modifications. That most commonly used is Gunning's. For all modifications it is necessary to have combustion flasks of Jena glass of about 250 to 300 c.c. capacity, and an ordinary distilling apparatus with a good cooling jacket (see Fig. 21, C). To the urine, in amount varying from 5 to 20 c.c. according to its concentration, are added 15 c.c. of pure concentrated sulphuric acid, 10 gms. of potassium sulphate, and about 1 gm. of copper sulphate. This, supported on a sheet of asbestos gauze, is then boiled over a free flame in a hood with a good draft until the fluid is a clear blue. The worker should be careful, if it is necessary to wash down

<sup>21</sup> Slemons, Johns Hopkins Hosp. Rep., vol. xii, 1904.

\* Personal communication.

the carbon from the sides of the glass by shaking the fluid, that he does not burn himself with this exceedingly hot acid. After the fluid is perfectly blue the heat should be continued for a few minutes or even half an hour, that the combustion may be perfect. Uric acid and other bodies are perfectly oxidized only after at least half an hour's further heating of the clear fluid. By this means practically all of the nitrogen has been converted into ammonia, and is hence present as ammonium sulphate. The oxidation may be aided by adding a little  $\text{KMnO}_4$ . The fluid is allowed to cool perfectly, distilled water is then added in excess, and the fluid poured into a distilling flask (see Fig. 21, A) of 1 litre capacity, with long neck and round bottom. The combustion flask is well washed into this flask, rinsing it three or four times with distilled water. Talcum powder or zinc granules may be added to prevent bumping. An amount of strong sodium hydroxide, specific gravity 1.230, found by previous experiments sufficient to more than neutralize the acid, is now added and the flask at once fitted to the Liebig cooler. The lower end of this cooler ends in a bent tube which vertically descends to the bottom of a small Erlenmeyer flask, D, of about 300 cc. capacity, in which have been put previously just 50 cc. of fourth-normal  $\text{H}_2\text{SO}_4$ . In the subsequent distillation, therefore, all the ammonia, both that given off at once in the cold and that on boiling, bubbles through this acid and is thus caught. The distillation is continued until about 100 cc. of distillate have passed over, but the boiling should never be too vigorous, and the apparatus should be watched to be sure no acid spurts into the cooler; hence the vertical tube has a safety-bulb, B, to prevent this. The Erlenmeyer flask may then be lowered and the distillate tested with lacmoid paper, to make sure that the ammonia has entirely passed over. The acid clinging to the end of the tube is washed into the flask. This sulphuric acid is then titrated against fourth-normal  $\text{NaOH}$ , using cochineal, methyl orange or pure litmus as indicator. There can be no doubt that the pure litmus is the best if the necessary precautions are used. The most convenient is cochineal, which can be used in artificial light as well, and is sufficiently correct for ordinary work. (The cochineal bugs are ground fine and extracted with 50 per cent. alcohol. The filtered extract is used as indicator.) From the 50 cc. are subtracted the number of cubic centimetres of fourth-normal  $\text{NaOH}$ , and the difference indicates the amount of fourth-normal  $\text{H}_2\text{SO}_4$  neutralized by ammonia. This value multiplied by 0.0035 gm. would equal in grammes the weight of nitrogen in the amount of urine used.

(NOTE.—This method does not indicate nitrates or nitro-compounds.)

In many laboratories the nitrogen of the urine is determined

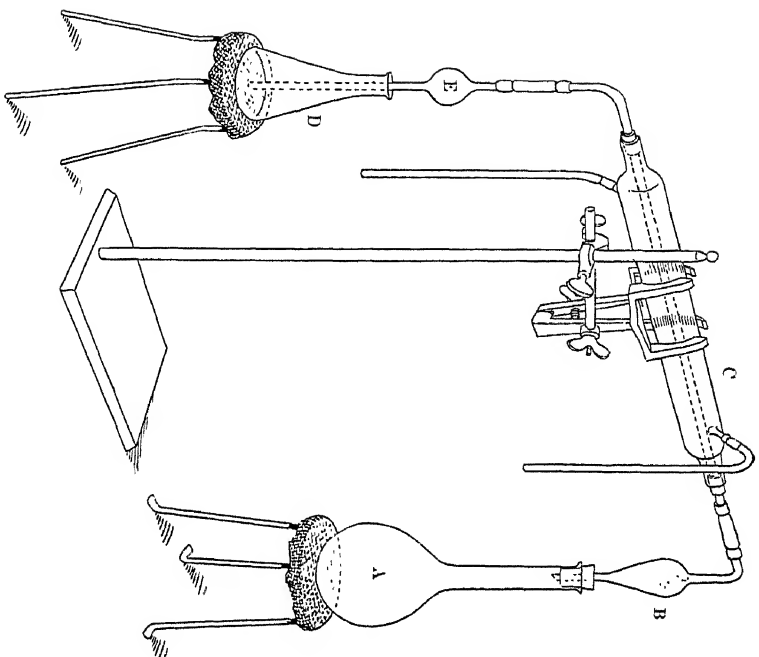


FIG. 21.—Distilling apparatus for nitrogen determination (Kjeldahl). A, distillation flask; B, safety bulb; C, Liebig cooler; D, Erlenmeyer flask to receive distillate and containing the standard acid; E, safety bulb to prevent back-flow.

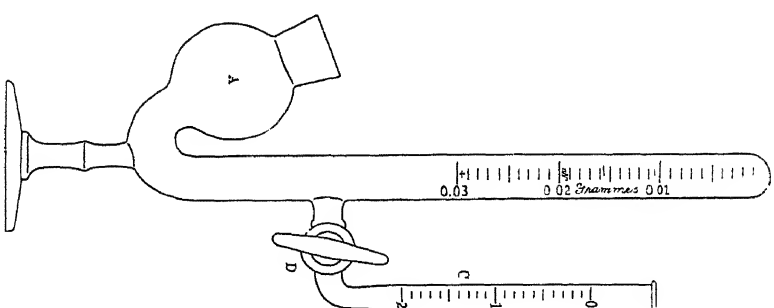


FIG. 21A.—Heinze modification of Hufner apparatus for urea determination. A, bulb; B, graduated tube to collect and measure the nitrogen; C, tube for urine; D, stopcock.



without distillation. The contents of the combustion flask are poured, and then the rinsings washed, into an ammonia-determination apparatus, the alkali added, and the determination continued as for ammonia (see page 127).

For the relation between CARBON AND NITROGEN, see Richardson.<sup>22</sup>

Urea is the chief nitrogenous body of the urine and the one which until recently has attracted most attention. The methods employed to determine it (Liebig's, Hüfner's, *et al.*) have not given correct results. The first, in fact, gives a fair nitrogen determination, and the second, a result fairly correct in some, but very incorrect in other cases.

The amount of urea as an index of nitrogen metabolism has been used as a test of the digestion. In this case a meal containing an excess of nitrogen is given, for illustration, 500 gms. of meat, eight eggs, and 200 gms. of bread; during this and the following day at least 50 gms. of urea should be excreted.

The amount of urea excreted by a normal person on an average diet varies from 20 to 40 gms., more in the case of men than of women. On a poor diet it may be from 15 to 20 gms., while on a very rich diet figures as high as 100 gms. in twenty-four hours have been reported. In general it may be said that for a vigorous person on an average diet about 30 gms. may be expected; for an invalid, about 20 gms.

The urea may be diminished because the nitrogen is diminished, or is excreted in other forms, particularly as ammonia. One of the most important functions of the liver is that of changing ammonia to urea; hence in certain cases of liver disease the output of urea diminishes and that of ammonia increases, constituting even 50 to 60 per cent. of the total nitrogen (see page 119). It is true, however, that in other cases with marked gross lesion of the liver the percentage is about normal. Again, the nitrogen may be eliminated as ammonia because of acids ingested or formed within the body and neutralized by it to protect the mineral alkali; hence it is withdrawn from urea formation. Such is true in diabetes and in cachexia, in which there is a disturbance of the carbohydrate absorption or use, thus forcing the body to use only the pure proteid of food or tissues,—that is, an acid-producing diet.

But the interesting question now is, Why is there any urea in the urine? There are various opinions on this point. One is that urea is the chief nitrogenous ash of nitrogenous food, and that a normal American on an "average" American diet will excrete from 20 to 30 gm. of it each day. Another opinion is that the urea represents that part of our nitrogenous intake which is over and above that which we really needed, and that the man "living rationally" would have very

<sup>22</sup> Am. Jour. Med. Sci., 1902, vol. cxxiv.

little urea in his urine. Another view is not so radical: It is that in protein digestion the split products are perhaps not resynthesized to protein, but the cleavage liberates the carbonaceous portion of the protein molecule which is used, while the nitrogenous portion is not used and is finally excreted as urea. One quite certain fact is that urea is the only nitrogenous ash which is diminished absolutely and relatively when the total nitrogen output is diminished, and that a man can keep in nitrogenous equilibrium and in good (?) health on an astonishingly limited diet (see the table on page 109).

ESTIMATION OF UREA.—*The Mörner-Sjoqvist*.—This method was thought reliable for the determination of urea alone. Albumin must first be removed by heat and acetic acid, and then the original volume of urine restored. Five cc. of urine are placed in a flask with 5 cc. of a barium mixture (saturated barium chloride solution to which barium hydroxide is added till 5 per cent. in amount); 100 cc. of a mixture of 97 per cent. alcohol, 2 parts, and ether, 1 part, are then added. This is allowed to stand until the next day in a closed vessel. It is then filtered on a suction filter, preferably through a small filter paper about 10 cm. in diameter and into a Kjeldahl flask. The precipitate is well washed with alcohol and ether. It is sufficient to obtain about 50 cc. of filtrate. Urea will be practically the only nitrogenous body left in solution. The alcohol and ether are then evaporated off at a temperature not above 60° C., or distilled off at reduced pressure if a good suction pump is at hand. When about 25 cc. are left there is added a little water and burnt magnesium oxide and the evaporation or distillation continued until the distillate is no longer alkaline, which is true, as a rule, when only from 10 to 15 cc. are left; otherwise ammonia will be left in the filtrate. The fluid is then poured—and the flask washed—into a flask. A few drops of concentrated sulphuric acid should then be added, and it is then evaporated on a water-bath to a very small volume, since we wish to get rid of all the alcohol which will blacken the solution and foam badly. Twenty cc. of pure sulphuric acid are then added and one continues as in the Kjeldahl nitrogen determination.

The amount of nitrogen multiplied by 2.143 will give the weight of the urea. Hoppe-Seyler advises that when the fluid is evaporated to 10 to 15 cc. about 10 gms. of crystalline phosphoric acid be added, and one proceed as in the Schön-dorff method.

A still better method is that of *Schöndorf*,<sup>25</sup> which gives results from 0.08 to 0.19 per cent. lower than by the Mörner. This method is based on experiments that phosphotungstic acid will precipitate all of the nitrogenous bodies excepting urea; it would precipitate this in a solution of over 3 per cent. urea.

Fifty cubic centimetres of urine (if the specific gravity be over 1017, it should be diluted) are measured in a closed graduated cylinder of 200 cc. capacity, and the acid mixture (phosphotungstic acid 10 per cent. 9 parts, HCl specific gravity 1.124, 1 part) added until full precipitation is obtained. The amount necessary for this should be determined by the following preliminary test. To 10 cc. of urine is added the phosphotungstic acid mixture from a pipette, stirring by shaking, until 1 cc. of the clear filtrate (filter repeatedly until clear) does not cloud when a few more drops are added. It is sufficient to get within 1 cc. of the necessary amount.

The cylinder is then filled up to the 150 or 200 cc. point with HCl (of sp. gr. 1.124, diluted ten times), well shaken, and allowed to stand for twenty-four hours. It is then filtered through a double filter

<sup>25</sup> Pfüger's Arch., Bd. 62.

until clear. The clear filtrate is rubbed up with  $\text{Ca}(\text{OH})_2$  until alkaline, and after the blue color disappears is filtered. Fifteen or twenty cubic centimetres (that is, the amount which corresponds to 5 cc. of urine) are then measured into an Erlenmeyer flask in which are put 10 gms. of crystalline phosphoric acid. This is heated in a dry chamber (a sand-bath) for four and a half hours, the time reckoned from the point when all the water is evaporated off, and at a temperature of  $150^\circ \text{C}$ . After cooling, the syrupy mass is dissolved in warm water, put into a distillation flask, and the further steps are the same as those of the Kjeldahl nitrogen determination. The amount of nitrogen multiplied by 2.143 will equal the weight of urea in 5 cc. of the urine.

Pflüger and Bleibtren advise that 5 cc. of the filtrate, after the addition of  $\text{Ca}(\text{OH})_2$ , be used to make an ammonia determination, the nitrogen of which is subtracted from the result; but if pure phosphotungstic acid be used this is unnecessary since the ammonia will all be in the precipitate (Gullich).

In all methods it is to be supposed other bodies nearly related to urea are determined as well. It has been repeatedly shown<sup>26</sup> that if there be even 0.1 per cent. sugar in the urine there is a considerable loss of urea nitrogen, hence all must be removed by fermentation.

*Folin Method.*<sup>26</sup>—To 5 cc. of urine in a 200 cc. Erlenmeyer flask are added 5 cc. concentrated  $\text{HCl}$ , 20 gms.  $\text{MgCl}_2$ , a piece of paraffin the size of a small hazel-nut, and 2 to 3 drops of 1 per cent. aqueous alizarin red. This flask, protected by a special safety-tube, is boiled till each drop of reflow makes a very perceptible thump; the heat is then reduced and continued one-half hour. The contents of the flask must not turn alkaline, hence when seen to turn red a little of the acid distillate is allowed to flow back from the safety-tube. At the end of an hour the contents of the flask are transferred to a 1 litre flask with about 700 cc. water, then about 20 cc. of 10 per cent.  $\text{NaOH}$  added and then distilled until the last trace of ammonia has passed over. This will take nearly an hour and the flask will be nearly dry. The distillate is then boiled to drive off the  $\text{CO}_2$ , cooled, and titrated. The ammonia of the urine and of the  $\text{MgCl}_2$  must be subtracted.

*Hüfner's Method.*—Formerly this was the most popular method for the quantitative determination of urea. Now, because of its ease of application, it is used in cases in which the nitrogen distribution is assumed to be approximately normal, in following the daily output of urea in a given case, and by the surgeons in comparing the separated urines obtained from the two kidneys through ureteral catheters. It is only an approximate method and open to so serious objections that it cannot be used in accurate metabolism work, or for careful diagnostic or prognostic observations. (For instance, if much ammonia be present in the urine, the amount of urea indicated by this method is not rarely twice the amount there could be were all the nitrogen present [determined accurately by Kjeldahl's method] contained in urea.)

The method is not accurate enough to warrant the use of Hüfner's expensive apparatus, and so simpler models are used. The simplest practical apparatus used is represented in Fig. 21, *a*. The

<sup>26</sup> Landau, Maly's Jahresb., vol. xxxiii.

bulb A is filled with a solution of sodium hypobromite which is made fresh for each determination by mixing equal quantities of the following two solutions:

Solution A. NaOH, 100 gm., dissolved in 250 cc. H<sub>2</sub>O.

Solution B. Bromide, 1 part; K.Br, 1 part; water 8 parts.

These solutions are stable if kept separate. After the bulb is filled the apparatus is inverted and manipulated until the fluid has replaced all the air in the tube B, and the bend connecting B with A is so full of this fluid that the tube will remain full when the apparatus stands erect. Urine is poured into the side tube, C, and the height of the column of urine is read. The stopper D is then turned, and exactly 1 c.c. of urine is allowed to flow into the tube B. The sodium hypobromite will decompose the urea (really, all the easily decomposed nitrogenous bodies), setting free most of its nitrogen. This will collect at the top of the tube B, and its volume can be read off by the scale, which also indicates the quantity of urea per 1 c.c. of urine which each volume of free nitrogen represents.

The Doremus tube is similar and yet is simple. It has no "foot" and no side-tube. The tube is held in the hand while the urine is introduced into B through A by a curved pipette holding 1 cc.

Using the Schlösing method, v. Jaksch<sup>27</sup> has shown that among patients in general, 83.93 to 91.07 per cent. of the total nitrogen is urea, and this constitutes from 95.85 to 98.36 per cent. of the nitrogen not precipitated by phosphotungstic acid; from 1.52 to 3.61 per cent. of the total nitrogen is in amido-acids, and from 5.16 to 8.51 per cent. of the nitrogen precipitated by phosphotungstic acid is in amido-acids and ammonia bodies.

At this point we may suggest that simply because a person has a certain disease it does not mean that that case will necessarily show the changes in nitrogen distribution usually ascribed to that disease; for organs in general are functionally very sufficient despite disease until the disease reaches a point rendering them functionally insufficient, then the characteristic changes may suddenly develop.

Halpern,<sup>29</sup> using similar methods, found in nephritis, carcinoma, and inanition a relative decrease of urea; yet this was not constant, for in some cases he found normal figures, in others an increase of extractives and ammonia but not of amido-acids; in liver disease there was no relation between urea and the amido-acids, although the former fell; in blood diseases, leukæmia, severe pernicious anæmia, and in tuberculosis, the distribution of nitrogen was normal. From the above results we see that in the usual run of cases the various diseases studied do not disturb the nitrogen distribution in any char-

<sup>27</sup> Zeitschr. f. physiol. Chem., 1901, xxxii, p. 504; 1902, xxxvi.

<sup>28</sup> Ibid, p. 355.



acteristic way. We must wait for observations on a series of more severe cases of these same diseases.

We give herewith a few of the *properties of and tests for urea*, since this is a most important body. Urea when pure occurs in crystals which are needles or prisms belonging to the tetragonal system; colorless, striated, pale, four-sided columns with ends of one or two oblique planes, and sometimes hollow. They contain no water of crystallization. It is not hygroscopic, and does not change in the air; it is decomposed by heat, the decomposition and the evolution of ammonia beginning at  $100^{\circ}$  C., but chiefly at  $130^{\circ}$  to  $132^{\circ}$  C.

The *furfural* test is one of the most important. According to Schiff, one crystal, the size of the head of a pin, is brought in contact in a porcelain dish with one drop of concentrated aqueous solution of furfural. At once is added one drop of hydrochloric acid (specific gravity 1.100) and one sees a rapid change of colors from yellow, to green, to blue, to violet, and in a few minutes a fine purple-violet color. Alantoin gives the same test, but less intense and slower. An old furfural solution will also give the test without urea. Huppert advises the following method: 2 cc. of concentrated furfural solution plus 4 to 6 drops of concentrated hydrochloric acid are mixed. The mixture must not stain red. To this is added one crystal of urea. In a few minutes is seen a deep violet color, which gradually becomes black, and then appears a black precipitate.

The *biuret* test is one of the best-known urea tests. Urea, if fused, gives off biuret and cyanuric acid. This occurs at a temperature of  $100^{\circ}$  C. To test this a few crystals are put in a dry test-tube and heated gently until fluid. This is then cooled, dissolved in water, made strongly alkaline with NaOH, and then 2 per cent.  $\text{CuSO}_4$  solution added drop by drop. A beautiful violet color will result.

When only a crystal or so is at one's disposal, as, for instance, in the case of frost upon the skin, the best urea test is the *nitric acid* or *oxalic acid* test. Urea in the presence of concentrated nitric acid forms a compound,  $\text{CO}(\text{NH}_2)_2 \cdot \text{HNO}_3$ , in crystals, thin rhombs or hexagonal plates, which often overlap like shingles. They are colorless, and have acute angles. If they form slowly, large, thick, rhombic prisms are produced. These crystals heated volatilized without residue, an essential point in the test to exclude similar crystals of the heavy metals. No nitrous acid should be present in the nitric acid, since this in the cold will break up the urea, forming carbon dioxide, nitrogen, and water. To perform the test, one crystal or one drop of the concentrated solution is allowed to come in contact under the cover-glass with pure nitric acid. At the line of contact is seen the rapid formation of the above-described crystals. The urea must be in the concentration of at least 10 per cent.

Urea oxalate,  $2\text{CO}(\text{NH}_2)_2 \cdot \text{H}_2\text{C}_2\text{O}_4$ , is less soluble in water than the nitrate, and hence this test is preferred by many. It is performed in the same way as the nitric acid test. The crystals are rhombs, hexagons, or plates. It is well to dissolve the urea in the least amount of absolute alcohol, and to use a concentrated ether solution of oxalic acid, or, better still, an amyl alcohol solution of both.

To *isolate urea* from any solution the albumin is first removed. The urine, e.g., faintly acid is concentrated at a low temperature to a very small volume; nitric acid is then added in excess, the mixture being kept cool. The precipitate is filtered and pressed between filter paper. It is then dissolved in water and decomposed with barium carbonate, dried upon a water-bath, and the residue extracted with strong alcohol. The extract is decolorized if necessary with animal charcoal. Urea recrystallizes on cooling from the warm alcoholic solution. To determine it the Schöndorff method is applicable to albumin-free fluids.

**Uric acid** is a substance which has attracted an absurd amount of attention, and been the object of a great amount of careful work. The present status of opinion is that it is a specific oxidization product of the nuclein basis, and is increased only by an increase of these bodies

in the food, or an increased metabolism of tissue nuclei. Horbaczewski considered that this body is derived especially from the nuclei of leucocytes. Although this may in some degree be true, yet it probably explains but a small part. It is an interesting fact that in birds and certain reptiles the uric acid is the chief nitrogen compound of the excrementa; that in some carnivora (dogs and cats) it sometimes fails. In the herbivora it is always present, but only in traces, and in man it is present in a larger but still very varying amount. It has been shown also that the body has the ability to synthesize uric acid. If hypoxanthin be fed a patient, 50 per cent. will appear as uric acid. In the case of birds it is probable that just as in mammals the chief end product of nitrogen is urea, but this is synthesized to uric acid, while in mammals it is excreted unchanged; and that, lastly, if uric acid be fed to the body it will oxidize some of it; hence one is very wary in arguing from the amount in the urine to that found in the body. Recent work tends to show it an even more specific product of the nuclein bases than was supposed, and its output quantitatively related to these, although it is often delayed.

Uric acid, when pure, is a white powder of very small prisms or plates. It is difficultly soluble in boiling water and very little in cold. It is more soluble if not pure. Urea is its best solvent, and this in the urine can hold all the uric acid there in solution. It is insoluble in alcohol and ether; somewhat in hydrochloric acid and alkaline carbonates. The cold solution does not redden litmus. It reduces Fehling's solution when heated, but not bismuth solution. It is broken up by NaOBr, about 47.8 per cent. of its nitrogen being given off. The output may be said to vary normally from 0.2 to 1.25 gms., an average of 0.7 gm. in twenty-four hours, which represents from 1 to 2 per cent. of the total nitrogen. It is increased physiologically by an increase in the nucleins of the diet, sweetbreads being a favorite food to show this, since they increase it from 0.5 to 2 gms. in twenty-four hours. The maximum output occurs from three to five hours after a meal (that of the nitrogen in nine hours). There is a relatively large output in the newborn. In the adult the nitrogen of the uric acid is to nitrogen of the urea as 1 : 50 to 70, but in the case of the newborn as 1 : 13 to 14.

The amount varies considerably, particularly in different individuals. Burian and Schur have simplified the question greatly by showing that the uric acid output may be divided into two fractions,—the exogenous and the endogenous. By exogenous is meant the uric acid which is formed from the food directly; the endogenous, that part arising from the tissue proteid. This endogenous fraction is therefore the interesting fraction to consider, and in metabolism work involving it the patient should be on a diet—*e.g.*, of eggs and milk—which

covers his nitrogen and heat needs, but which does not contain nucleins.

Concerning the pathological variations there is the widest divergence of opinion, and hardly one claim is unchallenged. This is chiefly due to the fact that the difference between the endogenous and exogenous was not recognized.

The uric acid is pathologically increased when there is an increased proteid catabolism. Such is true of fever, in which case the increase is parallel to that of urea.

There is an absolute increase in leukæmia, the record being that of Magnus-Levy's case, with an output of 8 gms. in twenty-four hours. As a rule, it is about 2 gms., and the nitrogen of the acid is to the nitrogen of the urea as 1 : 9.

The relation in gout is still uncertain. One thing is quite certain, that during the quiescent interval between attacks the acid is below normal, rising to normal with the acute symptoms, then to sink again. This is of diagnostic importance in a suspicious case of arthritis. Whether it is retention of the acid or diminished formation followed by an increase is still to be settled, but the large accumulations of the acid in the tophi and around the joints is good evidence of an increased production; these patients do not respond as normally to an increased nuclein-rich diet by an increased uric acid output.<sup>29</sup> In rheumatism the question is still unsettled. In diabetes mellitus the increase is not marked, 2 to 3 gms., and is due to diet; in pernicious anæmia an increase is claimed. In pneumonia during resolution the output is increased, probably from the breaking down of the nuclei in a large exudate. In cirrhosis of the liver it is said to be very much increased, Chabrié even stating that in certain cases the maximum, even 8 gms., is excreted. This is rather interesting, since the liver is certainly an organ which can synthesize uric acid. The uric acid diathesis so emphasized by Haig is still in dispute. V. Jaksch thinks it exists, symptoms of hypochondriasis and increased uric acid output being the two features.

On the other hand others have found that the output of uric acid is diminished by a poor diet, in nephritis, during the acute attack of gout, in certain chronic diseases, and after large doses of quinine.

One point of interest is that when the alloxuric bases are increased the uric acid decreases in the same proportion.

URATES.—The possible urates are:

(1) Neutral,  $M\bar{U}$ , which do not occur in nature.

(2) The monoacid or biurates,  $MH\bar{U}$ , which are gelatinous or crystalline bodies, and the best illustration of which are the needles found in tophi in gout.

<sup>29</sup> Reach, Münch. med. Wochenschr., No. 29, 1902.

(3) Quadriurates,  $MH\bar{U}\bar{U}$ , which are easily split to  $MH\bar{U}$  and  $\bar{U}$  by water, heat, or acid. They are less soluble than the biurates. The urate sediment is supposed to consist of this. Many observers think Roberts's quadriurates are merely mixtures of sodium biurate and uric acid.

TESTS.—The murexid test is the one commonly used. The uric acid is dissolved in two drops of nitric acid. This is evaporated carefully to dryness, the residue being a beautiful red. Ammonia is then added and the color changes to a purple red. Had NaOH or KOH been used in place of the  $NH_4OH$ , the color would be more of a blue or bluish-violet. The color disappears rapidly on warming, an important point to differentiate certain other bodies. The test is more beautiful if evaporation is done over a water-bath, and if the ammonia be not directly added but placed in a small glass under a bell-jar near the residue; also if but little uric acid be used. If the residue be not red but only yellow, too little nitric acid has been added. More should be added and the evaporation repeated.

Guanin, xanthin, epiguanin, will also give this test, but these are excluded if the substance used was insoluble in an excess of HCl. In confirmation the color should be bleached by further heating, and the Fehling's reduction test tried with the body.

QUANTITATIVE DETERMINATION.—Only approximately this may be done by the Heller's albumin test, underlaying the urine with two-fifths volume of nitric acid. A cloudy ring appears above the line of separation. If the ring comes before five minutes, uric acid is increased; if after five minutes, it is decreased. The urine must be albumin-free.

The most correct method is the *Ludwig-Salkowsky*. This method, as slightly modified by Schmoll, is as follows: 240 cc. of urine are precipitated by 60 cc. of magnesium mixture (100 gms. of  $MgCl_2$  dissolved in water, plus  $NH_4OH$ , till it smells strongly of ammonia. To this is added  $NH_4Cl$  until the precipitate is just dissolved, and the whole made up to 1 litre in volume). The mixture is then filtered, 250 cc. of filtrate (equalling 200 cc. of urine) are used, and precipitated with from 10 to 15 cc. of silver nitrate solution (1 litre containing 26 gms. of  $AgNO_3$  and enough  $NH_4OH$  to dissolve the precipitate. The volume is then made up to 1 litre. This should be kept tightly corked in a dark bottle). The urine is then filtered, the precipitate washed with distilled water, and then brought into suspension in a litre of water made just acid with HCl. Three to 4 cc. of  $CuSO_4$  (10 per cent.) are then added and the mixture boiled. The silver salt is then decomposed by  $H_2S$  while hot and just acid. After entire decomposition it is boiled and filtered and the filtrate evaporated to 15 cc. Ten to 15 drops of HCl are then added, and it is allowed to stand from one to two hours or more. The crystals of uric acid are then filtered out on a very small filter, washed with water slightly acidified with HCl, not too long, so that at the end the total volume of wash water is not over 40 cc. and the nitrogen of the precipitate on the filter paper then determined by the Kjeldahl method. The result multiplied by 3 will be the weight of uric acid. Or, the determination may be made gravimetric. The crystallizing solution is allowed to stand over night, and then is collected in a Ludwig glass wool or asbestos filter with a ground glass stopper, which has already been

dried at  $110^{\circ}$  C. and weighed. The precipitate is washed upon this, washing at first with the filtrate and then with the smallest amount of water, then with alcohol, then with  $\text{CS}_2$  and ether, dried and weighed.

This method, although the most accurate and one which with the small modifications can be easily finished at the end of half a day, is still too difficult and demands too elaborate an apparatus to justify its use in general clinical chemistry. In a case of gout all that is necessary is to know whether the uric acid be much diminished or not, and a slightly less accurate method will suffice. Folin's modification of Hopkins's method is recommended.

**Folin's Method.**—To 300 cc. of urine are added 75 cc. of a uranium acetate reagent (consisting of 500 gms. of ammonium sulphate and 5 gms. uranium acetate dissolved in 650 cc. of water; 60 cc. of 10 per cent. acetic acid are then added and the whole made up to 1 litre). This solution is to remove the phosphates and certain bodies not well understood whose presence would in certain pathological cases disturb the accuracy of the method. The urine thus treated is well stirred and allowed to stand five minutes, and then filtered through a double folded filter. From the filtrate 125 cc. are measured into two beakers, each volume representing 100 cc. of urine. Five cubic centimetres of concentrated ammonia are added to each and the solution set aside until the next day. The clear fluid is then decanted through a filter, the precipitated ammonium urate is collected on the paper and washed with a 10 per cent. solution of ammonium sulphate. It should be washed until the filtrate is almost chlorine-free. In testing the filtrate of the washing for Cl with  $\text{AgNO}_3$ , a little  $\text{HNO}_3$  should be added. The filter paper is then pierced and the ammonium urate washed into a beaker, using about 100 cc. of water. Fifteen cc. of concentrated sulphuric acid are then added, and the solution titrated while still hot with a twentieth-normal  $\text{KMnO}_4$ , until the first blush of red is seen through the whole volume of fluid. This color need last but a few seconds. Each cubic centimetre of the reagent indicates 3.75 mg. of uric acid. A correction of 3 mg. per 100 cc. of urine it is necessary to add.

By a twentieth-normal  $\text{KMnO}_4$  is meant one of such concentration that 1 litre would contain 0.05 gm. of available oxygen to oxidize the uric acid. Hence 1.576 gms. of recrystallized  $\text{KMnO}_4$  are weighed into 1 litre of water. Since weighing is not sufficiently accurate, it is best to make a slightly more concentrated solution. This is boiled, which renders the solution more permanent. It is then titrated against a tenth-normal solution of oxalic acid (6.3 gms. per litre) or potassium tetraoxalate (8.41 gms. per litre). Ten cc. of the oxalic acid solution are diluted to 100 cc. with distilled water, and 15 cc. of concentrated sulphuric acid added to produce a temperature of about  $60^{\circ}$  C. The potassium permanganate is then added drop by drop until a uniform red color appears which lasts about thirty seconds. The permanganate solution is then diluted until 10 cc. of the oxalic acid require 20 cc. of the  $\text{KMnO}_4$  solution for the end reaction.

It is interesting that at the beginning of the titration the red remains longer than later. This is due to the fact that the combustion of the uric acid is much promoted by the increased percentage of the sulphate of manganese. The color

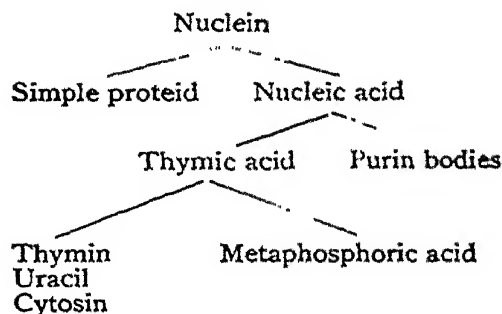
is not permanent, owing to other reducing bodies, and the student, to use the solution satisfactorily, should have standardized it himself, that he may know what to consider an end reaction.

To get an oxalic acid sufficiently pure it is necessary to recrystallize two to three times a cold saturated solution: or, better, to recrystallize first from hot dilute HCl (10 to 15 per cent.), then from hot alcohol, then from water. The aqueous solution must be heated till the odor of ethyl oxalate passes off. Oxalic acid cannot be dried in a desiccator or hot-air bath.

In the above methods great care must be used to avoid error from the uric acid or urates which may have precipitated, and which must be redissolved by warming the urine, or by the addition of a little saturated lithium carbonate solution.

Rudisch and Kleeberg<sup>30</sup> have recently reported a method for determining uric acid and the purin bases which they think even superior to the Ludwig-Salkowski method in accuracy, and so quick a method that it can be used clinically. These bodies are precipitated by an excess of fiftieth-normal  $\text{AgNO}_3$ , and the excess of silver determined volumetrically by fiftieth-normal KI, the end reaction being recognized by testing, after the addition of the successive portions of KI, the mixture in test-tubes with nitrous-sulphuric acid (25 cc.  $\text{H}_2\text{SO}_4$  to 75 cc.  $\text{H}_2\text{O}$ , then 1 cc. of fuming  $\text{HNO}_3$ ) and starch solution, until the blue of starch iodine compound appears. The separation of uric acid and the other purin bodies depends on the solubility of the silver compounds of the latter in strong ammonia solutions. This method is still recent, and for the details the reader is referred to the original article.

**The Purin Bases.**—The purin, alloxuric, xanthin, or nuclein bodies, all shown to contain the purin ring, occur in the urine in very small amount. Their formation from the nucleins is represented by the following diagram:



They are xanthin, guanin, hypoxanthin, adenin, heteroxanthin, paraxanthin, episarkin, epiguanin, methylxanthin, carnin. Of these ten bodies, the three which form the chief amount in the urine are heteroxanthin, paraxanthin, and methylxanthin, and these are derived wholly from the caffeine, theobromine, and theophyllin of the food. Guanin and carnin are still unproved. The total amount occurring in the urine is from 15.6 to 45.7 mg. in twenty-four hours. Others consider 87 mg. an average output for a mixed diet (Camerer), 44 mg. for a meat and 111 mg. for a vegetable diet.

**Xanthin** occurs normally in the urine only in minute traces. More rarely it is the chief constituent of urinary sediments and calculi, several of which have

<sup>30</sup> Amer. Jour. Med. Sci., 1904, vol. cxxviii. p. 899.

been described. It is increased in leukæmia, nephritis of children, in which case there may be 28.5 mg. per 100 cc. instead of, as normally, 3.8 mg. From 10,000 litres of urine 16 gms. have been isolated.

The principal test of xanthin is *Weidel's*. The body in question is boiled in a test-tube with hydrochloric acid and a little  $\text{KClO}_3$ . It is carefully evaporated to dryness, and the residue moistened with ammonia. A red or a purple-violet color results. Another test is to evaporate to dryness in a porcelain dish with nitric acid, producing a yellow residue which, on the addition of  $\text{NaOH}$  and warming, becomes a purple red.

**Guanin** is claimed to occur in the urine, especially in leukæmia. It gives the same nitric acid test as xanthin, excepting that the alkali gives a more blue-violet color. It does not give the Weidel reaction.

**Hypoxanthin** is present in the urine and in considerable amounts in leukæmia. It gives neither the nitric acid nor the Weidel tests.

**Adenin** occurs in urine, especially in leukemia. The characteristic reaction is that if the crystals be warmed slowly in an amount of water insufficient to dissolve them, at  $50^\circ \text{C}$ . there appears a sudden cloud. It does not give the nitric acid nor the Weidel test. Its other reactions are the same as hypoxanthin.

The QUANTITATIVE DETERMINATION used for these bodies is usually that of Salkowski. From 400 to 600 cc. of urine (albumin removed) are precipitated with a magnesium mixture and filtered. The filtrate is then precipitated with a 3 per cent. ammoniacal silver solution (6 cc. per 100 cc. of urine). The silver precipitate is washed thoroughly. It is then brought into about 600 to 800 cc. of water, slightly acidified with hydrochloric acid and decomposed with  $\text{H}_2\text{S}$ . The fluid is then heated to boiling and filtered hot. The filtrate is evaporated on a bath to dryness and the residue extracted with 3 per cent. hot sulphuric acid, from 25 to 30 cc. being used. This extract is allowed to stand for twenty-four hours. The uric acid is then filtered out and washed, the filtrate made alkaline and again precipitated with  $\text{AgNO}_3$ . It is then collected on a small chlorine free filter, washed, dried, carefully ashed, the ash dissolved in nitric acid and titrated for chlorine by the ordinary Volhardt method. One part of silver equals 0.277 parts of the xanthin base nitrogen, or 0.7381 parts of the xanthin bases. The uric acid can be determined in the same portion.

The enormous literature on the xanthin bases has lost its value since the methods formerly used have been found incorrect, hence at present nothing can be said of the clinical value of these bodies. It is interesting, however, that in leukæmia these bodies have been found to be increased, also in tuberculosis; and that there seems to be an antagonism between them and the uric acid, so that while the sum of both remains constant, when one increases the other decreases, and *vice versa*.

**Ammonia.**—The figures usually given for the twenty-four-hour output in normal urine of ammonia (from 0.3 to 1.2 gms., average 0.7 gm.) are considered by Taylor as much too high. He found that if the urine be carefully protected from all decomposition only about one-tenth that amount is present. The ammonia output reaches its maximum percentage during sleep—that is, when digestion is at rest. Many believe this ammonia is withheld from urea formation to balance acid ions, but this may not explain all, for there is still ammonia present after a long continued alkaline medication.

Ammonia is one of the most important products of proteid metabolism. In the arterial blood there is 0.4 mg., and in the portal blood 1.85 mg. in 100 c.c. (Hordynski). It is found in all the tissues, especially the stomach wall, which contains 36.4 mg., and in the in-



testinal wall 32.4 mg., per 100 gms. of the organ, being especially abundant at the height of digestion. In the other organs there is a more constant amount. It is rapidly changed to urea, especially by the liver, and hence in certain cases of disease,—e.g., cirrhosis and cancer,—with the total nitrogen unchanged, the percentage of urea will fall and that of ammonia rise. These ammonia bodies may be supposed to cause a certain toxæmia when increased, since injected ammonia compounds are toxic, and dogs with the Eck fistula manifest symptoms of toxæmia.

The relation of  $N:NH_3$  is quite constant on a constant diet, and is not affected by the amount of proteid. Much fat, however, does increase the percentage of  $NH_3$ . During secretion of the HCl of the gastric juice the nitrogen per cent. rises.<sup>31</sup>

Ammonia is increased by the ingestion of inorganic acids and of organic acids which cannot be further oxidized, and by those which arise in the body, man and carnivora thus protecting their native alkalinity against depletion in acid intoxication. The herbivora cannot protect themselves as well, and hence suffer more quickly. Such acids may arise in considerable amount in the normal body if the diet be strictly proteid. It is increased in oxygen starvation; in fever, during the febrile stage and continuing into the convalescence (Rumpf); in diabetes, in which case oxybutyric and perhaps diacetic are the acids present; ammonia may be present in diabetes in from 8 to 12 gms. in twenty-four hours and represent from 25 to 40.4 per cent. of the total nitrogen. In a case of periodic insanity Edsall found a marked reduction just before the attack, and a rise as the attack came on. In certain cases of liver cirrhosis the ammonia is increased, since the liver fails to form urea.

Dr. Williams has put the determination of ammonia to very practical use in his obstetrical wards of this hospital. In cases of the pernicious vomiting of pregnancy the percentage of ammonia is much increased, even to 20 to 45 per cent., while in the cases of nervous vomiting, or reflex from the pelvis, and in eclampsia, it is not. With this very high ammonia percentage the urine need show no casts or albumin. Definite hepatic lesions are found. If this high ammonia percentage is found, the uterus is emptied, and the ammonia drops at once. In a normal pregnancy the ammonia percentage is somewhat increased, reaching a maximum during labor.

**DETERMINATION.**—The Schlösing method is the one commonly used. (See Fig. 22.) This is simple, and yet is not perfectly satisfactory, since the results are somewhat too high. Twenty-five cc. of urine are mixed with 10 cc. of milk of lime. The broad vessel, B, in which this is placed, is at once covered over by a bell-jar, under

<sup>31</sup> See Schittenhelm, *Deutsch. Arch. f. klin. Med.*, 1903, Bd. 77, p. 517.



which have been previously put 20 cc. of tenth-normal  $\text{H}_2\text{SO}_4$ . The bell-jar is then well greased, to render it air-tight, and allowed to stand for from three to four days, during which time the milk of lime will have set free all of the ammonia which the sulphuric acid then takes up. It is well, that the sulphuric acid dish, C, rest upon the dish containing the urine. At the end of the three or four days the sulphuric acid is titrated against tenth-normal sodium hydroxide; the number of cubic centimetres multiplied by 1.7 mg. equals the weight of ammonia in 25 cc. of urine. If any moisture is present on the inside of the bell-jar the reaction of this should be tested, and if alkaline the entire interior of the bell-jar should be washed into the sulphuric acid before titration.

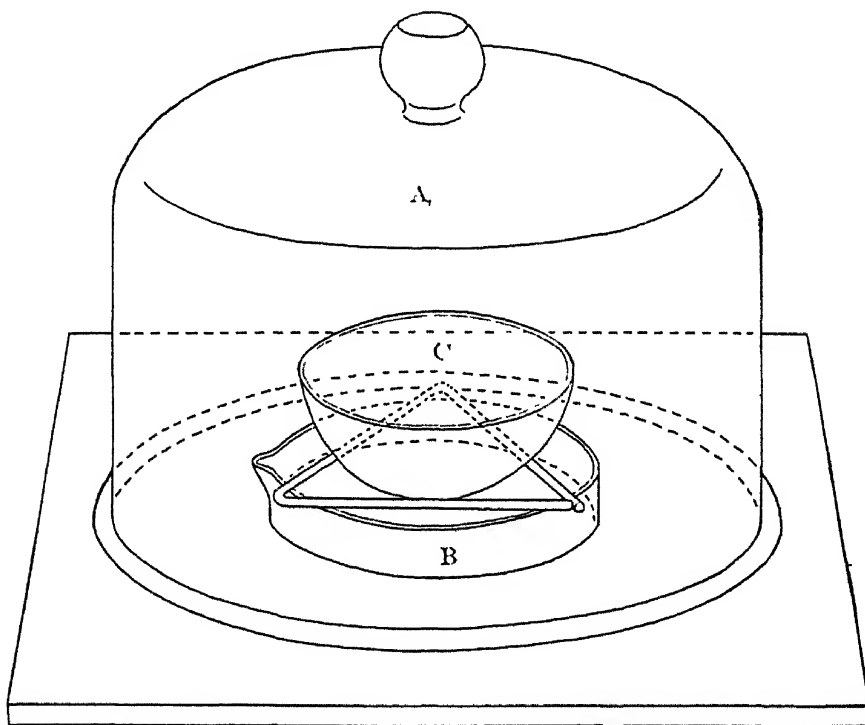


FIG. 22.—Ammonia determination, Schlösing method. A, bell-jar; B, dish containing urine; C, dish containing acid.

The modifications proposed by Schäffer, working under Folin's directions, are the following: To 25 cc. of filtered urine are added 0.5 gm. of sodium carbonate plus an excess of sodium chloride. The sodium carbonate will not split off ammonia from any of the other nitrogenous compounds, as for instance urea, and the sodium chloride will prevent decomposition. The urine should be placed on a dish from 15 to 17 cm. in diameter that the layer be not over 2 mm. deep; a wide crystallizing dish or a wide Petri's dish is the most satisfactory. The time may be reduced to forty-eight hours if the apparatus be kept at  $38^{\circ}\text{C}$ .

AMMONIA DETERMINATION.—*Folin's Method* (*Zeitsch. f. phys. Chem.*, 1902, xxxvii. p. 161).—For this method a special apparatus is used. The ammonia is liberated by sodium carbonate. No heat is necessary.

Twenty-five cubic centimetres of urine are measured into an aërometer cylinder (30 to 45 cm. high) and about a gram of dry sodium carbonate and from 5 to 10 cc. of crude petroleum (to prevent foaming) are added.

The upper end of the cylinder is then closed by a doubly perforated rubber stopper through which pass two glass tubes, only one of which is long enough to reach below the surface of the liquid. The shorter tube (about 10 cm. in length) is connected with a "calcium chloride tube" filled with cotton, which in turn is connected with a glass tube extending to the bottom of a wide mouth bottle (capacity about 500 cc.) which contains 20 cc. of  $N/10$   $H_2SO_4$ , 200 cc. of water, and the indicator. The special absorption bottle designed by Folin and pictured in Fig. 23 is very convenient and accurate, compelling a very intimate contact of the air from the cylinder with the acid in the absorption bottle. The absorption bottle is attached to a good filtering pump which can suck a very rapid air current. The air passing through the alkaline urine and then through the standard acid will in the course of about one and a half hours transfer every trace of ammonia to the acid. Its amount is then determined by direct titration with  $N/10$   $NaOH$ , using two drops of a 1 per cent. solution of alizarin-red as indicator and titrating to a red and not to a violet color.

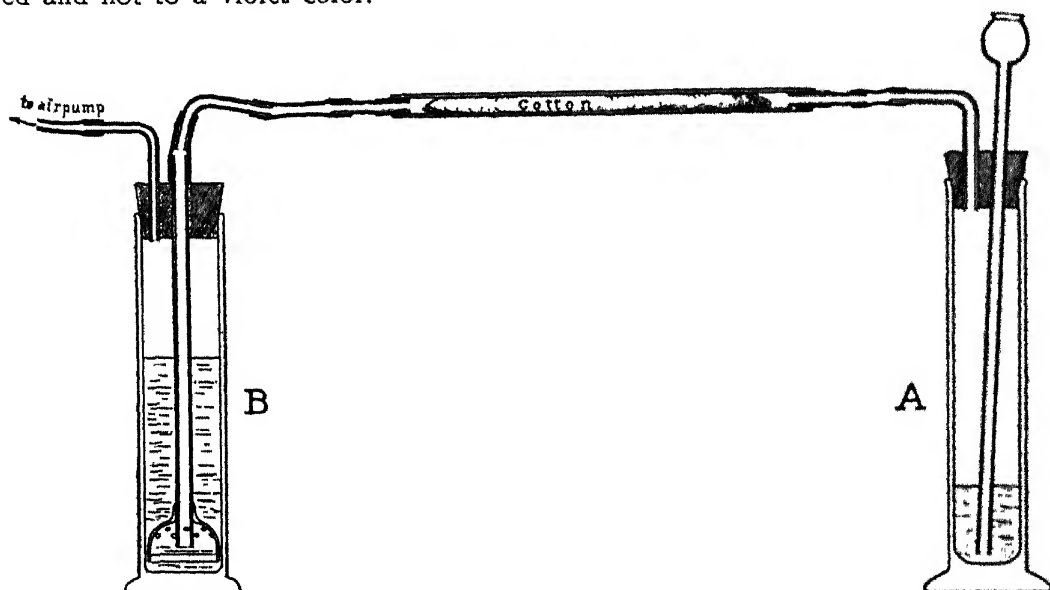


FIG. 23.—Folin's apparatus for ammonia and acetone determination. A, narrow tube for urine, connected by a tube containing cotton, with B, the cylinder containing acid.

In none of the titrations should phenolphthalein be used as indicator, since this fails for ammonium salts. Among those which may be used are alazarin red, cochineal, and a dilute solution of hæmatoxylin, which is used by Steyrer and seems very satisfactory.

**Creatinin**, the aldehyde of the creatin of muscle, occurs in the urine; creatin does not. In general its origin is the muscle of food and of the body. Its excretion is roughly parallel to that of urea; it is increased by a meat diet, and in hunger diminishes and even disappears. Sucklings have none in the urine until their diet is changed. It is probably increased by an increased metabolism of the body muscles. The relation between its output and muscular work has been much disputed, some claiming that it is increased only by excessive muscular work; others (Edsall) that it is increased by muscular exercise, and diminished in extensive muscular paralysis and in pathological conditions associated with a marked decrease in the function of

the muscles; that while it is not a perfect index of the condition of the muscular metabolism, yet it is the index nearest the truth. Normally the output is about 1 gm. a day. Folin (*Am. Jour. of Phys.*, 1905, vol. 13) has shown that the absolute quantity of creatinin eliminated in urine on a meat-free diet is a constant quantity, different for different individuals, but wholly independent of quantitative changes in the total amount of nitrogen eliminated. He found that moderately corpulent persons eliminated per 24 hours about 20 mg. creatinin per kilo of body weight, while lean persons eliminated about 25 mg. per kilo. Folin has also (*Festschrift f. Olaf Hammarsten*, iii, 1906) given good reasons for believing that biologically creatin and creatinin are not related; that creatinin is a waste product, and that creatin is treated as a food. Amberg and Morrill (*The Jour. of Biol. Chem.*, 1903, vol. iii, No. 4) found in the urine of the new-born a small but constant amount of creatinin.

*Jaffé's Test.*—To the urine is added a little aqueous solution of picric acid and a few drops of dilute NaOH. An intense red color appears at once at room temperature and, increasing, remains for hours. If acid be added it becomes yellow. Acetone should be removed by boiling, since it gives a more reddish-yellow color and is much fainter. Glucose gives on warming a red color. The test is positive in solution of 1 to 5000.

*Weyl's Test.*—To the urine are added a few drops of very weak sodium nitroprusside (sp. gr. 1003), then a few drops of weak NaOH. A ruby-red color appears which soon changes to yellow. Acetone will give a similar test and should be removed by heating, but the acetone urine would, if acetic acid be added, become of a cherry-red or purple-red, while in the case of creatinin the solution after adding acetic acid and heating becomes green and then a Berlin blue. The test is positive for 0.6 gm. per 1000.

The most important compound of creatinin is the zinc salt  $(C_4H_7N_3O)_2ZnCl_2$ . Creatinin is a reducing body, reducing Fehling's after long boiling to a colorless solution, and after still longer boiling, if an excess of copper be present, precipitates  $Cu_2(OH)_2$ . Creatinin, therefore, disturbs the copper sugar tests since it is a reducing agent, and, more important, it holds the  $Cu_2(OH)_2$  in solution. Bismuth is not reduced.

**QUANTITATIVE DETERMINATION.**—Salkowski's modification of Neubauer's method.—Of the urine, sugar- and albumin-free, 240 cc. are measured into a graduated cylinder, made faintly alkaline with milk of lime, carefully precipitated with calcium chloride and the whole then made up to 300 cc. After standing for 15 minutes this is filtered until 250 cc. of filtrate (equal to 200 cc. of urine) are obtained. This is faintly acidified with HCl and evaporated to about 20 cc. It is neutralized with soda, stirred up with 20 cc. of absolute alcohol, and then transferred, and the dish well washed with absolute alcohol into a 100 cc. measuring

flask. This is cooled, shaken from time to time, and then the flask is filled with alcohol to the 100 cc. mark. After standing for 24 hours the contents are filtered through a dry paper. Of the filtrate 80 cc. (which equals 100 cc. of urine) are mixed with 0.5 to 1 cc. of an alcoholic, absolutely acid-free  $\text{ZnCl}_2$  solution (sp. gr. 1.2). The covered beaker then stands in a cool place for 2 or 3 days. The precipitate is then collected on a small, dry, weighed filter paper, washed with as little alcohol as possible, dried at  $100^\circ \text{C}$ ., and weighed. One hundred parts of  $\text{ZnCl}_2$ -creatinin precipitate equal 62.44 parts of creatinin. Instead of weighing the nitrogen of the precipitate may be determined.

**CREATININ—FOLIN'S QUANTITATIVE METHOD** (*Am. Jour. of Phys.*, 1905, vol. 13, p. 48).—This determination is based on the color reaction which creatinin (and no other normal urinary constituent) gives with picric acid in alkaline solution. A high grade colorimeter is necessary, and Folin recommends that of Duboseq.

The reagents necessary are: a N/2 solution of potassium bichromate (which will contain 24.55 gm. per litre); a saturated picric acid solution (containing about 12 gm. per litre); a 10 per cent. solution of sodium hydrate.

Ten cubic centimetres of urine are measured into a 500 cc. volumetric flask, 15 cc. of the picric acid and 5 cc. of the sodium hydrate solutions are then added, and the mixture is allowed to stand for five or six minutes.

This interval is used to pour a little of the bichromate solution into each of the two cylinders of the colorimeter. The depth of the solution in one of the cylinders is then accurately adjusted to the 8 mm. mark. With the solution in the other cylinder a few preliminary colorimetric readings are made simply for the sake of insuring greater accuracy in the subsequent readings of the unknown solution. The two bichromate solutions must of course be equal in color, and in taking their readings no two should differ more than 0.1 mm. or 0.2 mm. from the true value (8 mm.) leaving out of consideration the very first reading made, which is sometimes less accurate. Four or more readings should be made in each case, and an average taken of all but the first. After a while one becomes sure of the true point, and can take the average of the first two readings.

At the end of five minutes the contents in the 500 cc. flask are diluted up to the 500 cc. mark. The bichromate solution is thoroughly rinsed out of one of the cylinders by means of the unknown solution and several colorimetric readings are then made at once.

The calculation of the result is very simple. It has been determined experimentally that 10 mg. of perfectly pure creatinin give under the conditions of the determination 500 cc. of a solution 8.1 mm. of which have exactly the same colorimetric value as 8 mm. of a N/2 bichromate solution. If then for example it takes 9.5 mm. of the unknown urine-picric solution to equal the 8 mm. of the

8.1  
bichromate, then the 10 cc. of urine contain  $10 \times \frac{8.1}{9.5} = 8.4 +$  mg. of creatinin.

If the 10 cc. of urine are found to contain more than 15 mg. or less than 5 mg. of creatinin the determination should be repeated using correspondingly different amounts of urine, since outside of these limits the determination is much less accurate.

The determination takes less than 15 minutes. Roberts (Bull. No. 66, Hygienic Lab., Wash., D. C., June, 1910) describes a simple colorimeter, adapted for field work, which seems very practical.

**Oxyproteinic and Alloxyproteinic Acids.**<sup>22</sup>—The first of these bodies was isolated by Gottlieb and Bondzynski, and the latter by Bondzynski and Panek. Although these bodies have not been sufficiently studied as yet, and already some have been unable to confirm this work, yet their presence in normal urine is claimed

<sup>22</sup> Bondzynski and Panek, Ber. d. d. chem. Gesell., 1902, vol. xxxv, p. 2959.

to be sufficient in amount to explain all or quite all of the neutral sulphur, which renders them very interesting. These writers, however, also think the oxyproteinic acid explains Ehrlich's Diazo reaction (see page 159). Their sulphur content is about 6 per cent. They stand the nearest to proteid of all the products of proteid metabolism, and yet give none of the proteid reactions. In amount of the alloxypoteinic acid are excreted about 1.2 gms. per day, the oxyproteinic acid in about three times that amount.

#### THE INORGANIC ACIDS AND BASES

**The Chlorides** are one of the most important groups of solids in point of amount found in the urine. Measured as sodium chloride, there are excreted in twenty-four hours from 10 to 15 gms., seldom more. Chlorine is present in inorganic salts, the little claimed in organic compounds being much disputed, but with the weight of evidence against it.<sup>34</sup>

The source of the chlorides is the food. The amount excreted depends in the first place upon the amount ingested. Starvation will reduce them to a trace. More is excreted during the day than during the night. Chlorine is increased by increasing the water output and by active exercise. It is diminished from loss of fluid by diarrhoea or by vomiting; also by transudate and exudate formation, and increased as these fluids are reabsorbed, provided the absorption be rapid. In fevers it is diminished in a remarkable way, especially toward the crisis, following which, its increase, Sahli considers, is as important a sign of improvement as the lowering of the temperature; in pneumonia some think the rise may be the first sign of improvement; its entire absence a serious sign. A great diminution or absence of chlorides in the urine in a doubtful fever strongly suggests pneumonia. After the crisis the output soon returns to normal. The explanation is not clear. The drop is not due to the diet, since an increase in the amount of chlorine ingested is not followed by a corresponding rise, as normally. Among the reasons given are, that during fever the catabolism is of those proteids poor in chlorine; but it is found that chlorides per mouth or injected subcutaneously are also retained in the body; others say that they are retained in the exudates present, or again with retained water; but the retention of the water is itself a much disputed point. Sahli considers that all these factors are present. A great deal of experiment has recently been done. There certainly is a definite retention of chlorides, but the reason is not the lack of absorption nor the food. The chlorine is not increased in the blood, but is accumulated in the other fluids of the body and in the tissues, being increased in the tissues of some cases with marked renal insufficiency to even four times the normal (Achard and Laubry). Van der Bergh<sup>35</sup> explains it as an attempt of the blood to maintain its osmotic tension, there

<sup>34</sup> Ville and Moitessier, *Compt.-rend. Soc. de Biol.*, liii. p. 673.

<sup>35</sup> *M. J.*, vol. xxxi.

being an accumulation of the products of metabolism in the plasma due to a slight insufficiency of the kidney, which increases the osmotic tension of the blood, hence the chlorides do not enter the circulation, but remain fixed in the tissues. After convalescence has begun there is a sudden return to normal which Achard and Laubry name a "chlorine crisis." The sulphates and the phosphates do not return to normal at the same time. To these chlorine crises is attributed a prognostic value.

We have examined the records of thirty-four cases of pneumonia in this hospital. It is our routine in almost every case of pneumonia (all on a pure milk diet, 1500 cc. q. d.) to determine the total amount of the chlorides daily. Six of these cases were with crisis. In two the chlorides showed a drop toward the crisis. In one case the crisis was preceded by a rise. In the other cases the rise began with, or even four or five days later than, the fall in temperature. In these very few cases it will be seen that we obtained very little prognostic value from the determination of the chlorides. In no case were the chlorides entirely absent. The average on the day before the crisis was 1.3 gms., varying from 0.7 to 2.1 gms. The greatest rise began on the fifth day after the crisis, on which day it varied from 3.8 to 4.9 gms.

Of twenty-two cases of lysis, in seven-tenths of the cases there was a drop toward lysis. In two-tenths the chlorides began to rise one to two days before the temperature began to fall. On the first day of the lysis in ten cases there was above 1 gm., an average of 2.6 gms., and in one case 9 gms. In three cases they were absent before defervescence, and in two cases during the fall of temperature, hence in these cases entire absence was not a bad sign. They were lowest during the drop in one-third, and just before the temperature began to fall in two-thirds of the cases. They began to rise with the lysis in just one-half of the cases. The chief rise began after the temperature had reached normal. It was then rapid.

In five fatal cases the chlorides fell steadily until the end in three and rose in one. In one case death was preceded by six days of entire absence of chlorides.

In one case of delayed resolution the chlorides were interesting. Nineteen determinations were made during a period of twenty-two days. The lowest amount was 4.3 gms., and this occurred after the lysis. For the most part they varied from about 5 to 10 gms. per day, hence in this case there was comparatively little retention.

In those cases in which the fall in temperature is succeeded by several days of very slight fever the chlorides do not rise until the temperature is about normal. In cases with a normal temperature but with a continuous slight leucocytosis they did not rise until this had fallen below 10,000.

After chloroform inhalation the chlorides are increased. In diabetes insipidus there is a marked increase with the polyuria. In all chronic diseases there is a decrease which may be due to disturbed absorption, or to the diet, or to the condition of the kidneys.

In gastric disease the chlorine is diminished when there is considerable vomiting; when absorption is diminished, as in malignant pyloric stricture; and when lost by lavage or diarrhoea.

In chronic diseases, if the output becomes as low as 2 gms., and the diet cannot explain this drop, it is an ominous sign, and the cessation of chlorine one of oncoming death. It is said to aid in the differen-

tial diagnosis between meningitis, in which the output is very low, and typhoid, where it is only moderately low. There is a marked diminution in cholera, pyæmia, puerperal fever, and acute articular rheumatism. In cirrhosis of the liver it is said to be increased.

The retention in nephritis has attracted especial attention, particularly in view of the recent work of Widal and others concerning œdema. Their explanation is that, given a slight renal insufficiency, there may be a specific retention of chlorides, the output of other solids remaining normal. These chlorides are retained by the tissues and there retain water, thus leading to œdema. By "chloruræmia" is meant a partial renal insufficiency for chlorine elimination, with a rapidly developing general œdema, low Cl output, and increased albumin in the urine. It is rather hard, on this basis, to explain the absence of œdema after even a week of total suppression of the urine due, *e.g.*, to calculus, or in those cases in which at operation the only functioning kidney is removed. The injection of physiological salt solution does not seem to cause œdema (perhaps since so dilute), and seems even to improve the condition of the case (Ferrannini), but if increased albumin, slight hæmaturia, and sometimes uræmic convulsions follow the injection immediately, we cannot consider the injection harmless. We have repeated this work with varying success, but with none if the water intake be also controlled. This amount of salt makes the patient very thirsty and he consumes much more water. Achard and Loeper found that if 10 gms. of sodium chloride be given per mouth in acute nephritis, little or none of the ingested chlorine is excreted, the chlorides remaining low, from 1 to 2 gms. per day. In subacute nephritis with 4 to 10 gms. before the dose there is a slight increase, while in interstitial nephritis with 2.8 to 3.4 gms. output the most of that given is excreted. In uræmic conditions there may be little or none excreted.

ESTIMATION.—A rough estimation of the amount of chlorides is made in the following way: To a test-tube of clear urine which contains no albumin 10 drops of pure nitric acid are added and then one drop of  $\text{AgNO}_3$  (1:8). If the chlorides are normal or increased the precipitate is a compact ball which sinks to the bottom. If diminished, this ball is less compact; if much diminished, until only a cloud is produced without solid flakes. If the last be true, that is, a cloud merely, it means a chloride content of 0.1 per cent. or less.

QUANTITATIVE DETERMINATION.—The best method is Arnold's modification of Volhardt's method. With the chlorides are estimated also the minute trace of cyanides. The principle upon which the test rests is the precipitation of hydrochloric acid by silver nitrate in a solution made strongly acid by nitric acid. An excess of silver chloride is added, and after the precipitate is filtered out the excess of silver is determined by titration with ammonium sulphocyanate. The urine should contain no nitrites, and most observers add also, no albumin or albumose, since these are precipitated as silver albuminates. If albumin be present, it may be necessary to ash the urine (Neubauer's method). Hammarsten recommends that the albumin be removed by boiling with a trace of acetic acid. If this be done, however, the precipitate must be washed for some time in order that the abundant chlorides retained in the precipitate may be regained.



Solutions necessary:

(1)  $\text{AgNO}_3$ . 1 cc. equals 10 mg. of  $\text{NaCl}$ . The pure crystalline  $\text{AgNO}_3$  is used, 1 litre to contain 29.075 gms. of the salt.

(2) Cold saturated solution of iron ammonium alum, or ferric sulphate, chlorine free (50 gms. of  $\text{Fe}_2\text{O}_3$  per litre).

(3)  $\text{HNO}_3$ . Specific gravity 1.2, chlorine-free. If chlorine be present the acid should be distilled. The nitrous acid should be removed by urea.

(4) An ammonium sulphocyanate solution, 10 cc. of which will equal 10 cc. of the silver nitrate solution. To obtain this, 12.9 gms. of the  $\text{NH}_4\text{SCN}$  are weighed and dissolved in a little less than one litre of water, and well mixed. Twenty cc. of the silver nitrate solution, 5 cc. of the iron alum, and 4 cc. of nitric acid are mixed in a flask and then diluted to 100 cc. The ammonium sulphocyanate solution is then added from a burette. The first precipitate is brown, which at once gives place to a white precipitate of silver cyanate; the brown ferric cyanate remains only after the last particle of silver has been precipitated. The end reaction is very sharp. The solution should then be diluted the necessary amount and the fluid again tested to make sure that 10 cc. of the silver nitrate solution equals 10 cc. of the ammonium sulphocyanate. Others recommend (v. Jaksch) that this latter solution be so made up that 25 cc. will equal 10 cc. of the silver nitrate, while others that 20 cc. equal 10 of the silver nitrate.

In Arnold's method 10 cc. of urine are carefully measured with a pipette into a flask on the neck of which is a 100 cc. mark. Then are added 20 to 30 drops of nitric acid and 2 cc. of the iron alum solution. If necessary a few drops of 8 per cent.  $\text{KMnO}_4$  are added until all red color disappears. The silver nitrate solution is then slowly run in, constantly shaking the flask until one is sure that all the chlorine has been precipitated and that there is an excess of silver. The flask is then allowed to stand for about ten minutes and then filled to the 100 cc. mark with water. This should then be mixed very thoroughly. There should be an excess of iron, otherwise the nitric acid can decolorize the ferric cyanate, but this excess of iron causes a brown rather than a red color in the end reaction. It is usually safe to add 20 cc. of the silver solution, while others recommend that 15 cc. be used. In general, a considerable excess gives the best results.

After the observer is sure that the contents of his 100 cc. flask is thoroughly mixed, it is then filtered through a dry filter until 50 cc. of clear filtrate are obtained. This is titrated with the ammonium sulphocyanate solution until the end reaction. The amount used indicates the excess of the silver solution in 50 cc. of filtrate. This amount multiplied by 2, since only one-half of the filtrate was used,



and subtracted from the number of cubic centimetres of silver nitrate originally added, will give the number of cubic centimetres of silver nitrate actually precipitated by the chlorides of the urine. This multiplied by 10 mg. will give the weight of the chlorine as sodium chloride in the amount of urine used.

Some add the iron alum solution to the 50 cc. of filtrate, not before. A much-jaundiced urine should be decolorized by adding a few drops of potassium permanganate and nitric acid. The urine is then warmed, allowed to stand for a few minutes, and filtered.

Harvey\* recommends a modification of the Volhard method which seems as accurate, and which surely is much simpler.

The solutions used are the silver solution described above, a sulphocyanate solution prepared as above, 20 c.c. of which are equivalent to 10 c.c. of the solution of silver nitrate, and a third solution, the "acidified indicator," which is prepared as follows: to 30 c.c. of water are added 70 c.c. of nitric acid (Sp. gr. 1.2, or 33 per cent.). One hundred gm. of crystalline ferric ammonium sulphate are dissolved in this menstruum, and the solution is then filtered.

Five cubic centimetres of the urine are pipetted into a small beaker and diluted with about 20 c.c. of distilled water. Next, the chlorides are precipitated with exactly 10 c.c. of the solution of silver nitrate, and about 2 c.c. of the acidified indicator are added. The solution of ammonium sulphocyanate is then run in from a burette until the first trace of red shows throughout the mixture. The number of cubic centimetres of the sulphocyanate solution used is divided by two (since 20 c.c. of this solution equal 10 c.c. of the silver solution), and this quotient is subtracted from 10. The difference is the amount of solution of silver nitrate used to precipitate the chloride in the urine. Each c.c. of this is equivalent to 0.01 gm. of NaCl.

**Phosphates.**—Phosphoric acid occurs in the urine of man in considerable amount, and is often encountered as the precipitate in an alkaline urine, a constituent of some of the most common crystals, and the principal ingredient of some of the commonest stones. In addition to the mineral phosphate there is always a little phosphorus in organic combination.

The amount weighed as  $P_2O_5$  in the urine of an adult is from 1 to 5 gms. in twenty-four hours, with an average of about 3.5 gms. The earthy phosphates are estimated as 1 to 1.5 gms., the alkaline from 2 to 4 gms. It varies chiefly with the food, especially with its content of calcium and magnesium, since these in the intestines form insoluble phosphates, which are little absorbed, hence the output may be less than one gramme. It is for this reason that in certain of the herbivora phosphoric acid is present only in a trace. It is important in metabolism experiments to control the diet carefully, that one may be sure

\* Arch. of Int. Med., July 15, 1910, vol. 6, p. 12.

that an approximately constant amount will be absorbed. Ehrström considers, however, that the calcium of the food is not as important as early studies led one to believe, and thinks that acid calcium phosphate can be absorbed in considerable amounts. Nevertheless, from the stools he could recover from 12 to 50 per cent., an average of 30 per cent., of the total phosphorus ingested.

The phosphates are increased by an increased metabolism of the body tissues, and also by a nuclein-rich diet. The amount from this source, however, is small. They are increased by hard muscular work. In starvation the phosphorus falls a little, yet more is excreted relative to the nitrogen, and in this condition the relative value, that is  $P_2O_5$  divided by N, equal 0.18. Ehrström found that the phosphorus was not excreted parallel to the nitrogen. In dogs on a pure meat diet the nitrogen is to the phosphoric acid as 8.1 : 1.

Clinically the phosphates have been the subject of much discussion. Some state that they are increased in extensive disease of bones, as rickets, osteomalacia, diffuse periostitis, etc., but concerning each of these diseases there is a great dispute. Some say they are increased in destructive disease of the lungs, especially early tuberculosis, but this also is open to considerable doubt, for the coincidence of disease and increased phosphoric acid output may be accidental. The same may be said of extensive disease of the nervous system. In mental disease Folin and Schäfer<sup>36</sup> found that during the periods of excitement the relative amount of phosphoric acid was diminished, but absolutely there was little change. They consider that the phosphorus metabolism of the brain is disturbed on the excited days, and that there is a compensatory increase on good days. It is also increased in meningitis, yellow atrophy of the liver, in diabetes mellitus and insipidus, after the use of chloral, KBr, and lastly in phosphorus poisoning.

They have been found diminished in acute diseases, for instance in pneumonia, during the height of the fever: this is true especially of the earthy phosphates, which point Gouraud considers may aid in the differential diagnosis between tuberculous processes, in which case the earthy phosphates are increased, and pneumonia. At the crisis comes a sharp rise, but one not always simultaneous with the rise in nitrogen and chlorine, while the ratio between the earthy and the total phosphates increases considerably. In one case of typhoid fever the total  $P_2O_5$  rose after defervescence from 1.5 to 13 gms. The output of phosphoric acid in fevers is not at all parallel to that of chlorine, and there occur sudden large outputs which are independent of the diet (v. Jaksch has found, however, that in the acute lobar pneumonia of children there may be increased phosphoric acid).

<sup>36</sup> Amer. Jour. Phys., vol. vii. p. 135.

They are diminished in most chronic diseases; in all renal diseases, due it is supposed to the renal insufficiency, Purdy stating that the diminution in phosphates is almost as constant a feature as albuminuria; in pregnancy, in which case it is attributed to the fetal bone formation; and in gout, in which disease the line of phosphoric acid runs quite parallel to that of uric acid. Certain cases have been reported in which, without any sugar output but with all the symptoms of diabetes mellitus, there is a phosphate excretion of even as high as 10 gms. in twenty-four hours. Such are cases of the so-called "phosphatic diabetes." To deserve this name the output should be at least from 3.5 to 4 gms. per day. Teissier claims that these cases resemble diabetes, while others say they more nearly resemble neurasthenia. In some cases this is simply a temporary absence of the sugar which later appears, and as the phosphates fall.<sup>37</sup> A relative increase, formerly also passing under this name, in which  $P_2O_5:N :: 17$  to  $20 : 100$ , occurs in malnutrition and starvation.

(For phosphaturia, see page 105.)

The organic phosphorus has been found by Mandel and Oertel not to be influenced by a phosphorus rich diet. They considered that its output is a good index of tissue catabolism.

There are four groups of phosphate salts,—the diacid, monacid, normal, and basic,—the salts varying in solubility in the order in which they are stated, the diacid being the most soluble. The monacid salts of calcium and magnesia are precipitated when the urine is made alkaline. On heating the urine, a flocculent precipitate of the normal salts is often seen (basic, v. Jaksch), which must not be confused with the albumin cloud. It has been found that this precipitate on heating is always the calcium phosphate with a trace of  $CaOx$  and  $CaSO_4$ , but never magnesium, since the calcium salts are more insoluble than the magnesium salts.

In leukæmia White and Hopkins<sup>38</sup> have found an absolute and a relatively (to nitrogen) diminished output, and they suggest a retention of the phosphorus in the blood to build new leucocytes. In the new-born the proportion between nitrogen and phosphoric acid is from 5 to 8 : 1.

Of the normal phosphates that of greatest interest is the  $MgNH_4PO_4 \cdot 6H_2O$  in the beautiful coffin-lid crystals of triple phosphate, which occur in all alkaline or amphoteric urines containing enough ammonia.

The acidity of the urine, although due to many acid components and to an unknown degree to each, is, however, chiefly due to the phosphates. Normally 60 per cent. of the phosphoric acid is present

<sup>37</sup> See Ralfe, *Lancet*, March 5, 1887.

<sup>38</sup> *Journal of Physiology*, vol. xxiv. p. 42.

as diacidphosphate, and 40 per cent. as the monacid salts, but the former varies from 34.9 to 74.2 per cent. In general it may be said that the urine is amphoteric if the diacid salts are from 30 to 50 per cent. and the monacid from 70 to 50 per cent. of the whole.

A common test, allowing an approximate determination of the phosphates, is made by filling a test-tube half full of filtered urine, adding ammonia, warming, and then allowing it to stand. If in from eighteen to twenty-four hours the deposit is from one-fourth to one-half inch deep the amount is normal, if less it is diminished. This is a precipitate of earthy phosphates. These are then filtered away, all of the filtrate put in the test-tube, and one finger's breadth of magnesium mixture added. The urine is then warmed and the precipitate of alkaline phosphates allowed to settle. If during the same length of time the sediment is from one-half to three-fourths inch deep the amount is normal.

The urine may be cleared of phosphates by precipitation with basic or neutral lead acetate.

#### QUANTITATIVE DETERMINATION.—Uranium nitrate method.

Phosphoric acid as a diacid salt is precipitated by uranium nitrate, and if cochineal be used as indicator the first excess of the uranium salt will give with it a green compound which serves as the end reaction. Uranium nitrate is preferable to the acetate, since its solutions are more stable, but even the nitrate is none too stable, and should be frequently restandardized. Since free nitric acid is liberated in the reaction, and this will dissolve a certain amount of uranium phosphate, sodium acetate is added in excess; and that all the phosphoric acid may be present as a diacid salt, acetic acid as well. The boiling urine should be titrated, since the end reaction is quicker and sharper, giving a more decided green.

Neubauer recommends that for greater accuracy the urine be precipitated with magnesium mixture and the precipitate washed on a small filter with dilute ammonia (water, 3 vols., 10 per cent.  $\text{NH}_4\text{OH}$ , 1 vol.). The precipitate is then dissolved in acetic acid, diluted to 50 cc. with water, and the titration continued as with the urine. The results obtained are somewhat lower.

Albumin and sugar may be present. The titer changes with the volume of reagent used. For instance, if 20 cc. are used, 1 cc. will indicate 4.98 mg.  $\text{P}_2\text{O}_5$ ; 21 cc., 5 mg.; 40 cc., 5.14 mg. Hence the uranium nitrate fluid should be standardized against a phosphoric acid solution of about the concentration of normal urine.

The fluids necessary are, 1, a phosphate solution 50 cc. of which contain 0.1 gm. of  $\text{P}_2\text{O}_5$ . This is so difficult to prepare that we recommend that it be purchased from those chemists who make a specialty of such work. This is the standard solution.

2. A solution containing 100 gms.  $\text{NaAc}$  and 30 gms. acetic acid in 1 litre of water. Five cc. of this fluid added to 50 cc. of urine will keep all the phosphates in the diacid condition and prevent the presence of free nitric acid.

3. An alcohol cochineal extract; the ground cochineal insects digested in 25 per cent. alcohol.

4. Uranium nitrate solution, 1 litre of which contains 35.461 gms. of  $\text{UO}_2(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$ . This solution is standardized against solution 1. Three gms. of NaAc are added, since this salt always contains some free nitric acid. One cc. of this solution will indicate 5 mg.  $\text{P}_2\text{O}_5$ , hence 20 cc. should give the end reaction with exactly 50 cc. of solution 1.

To 50 cc. of the standard phosphate solution are added 5 cc. of solution 2, then a few drops of the cochineal tincture. The amount of indicator added is of moment, and a rather strong solution is desirable. This in an Erlenmeyer flask is brought to the boiling point. The uranium solution is then added to the boiling fluid in small amounts, shaking constantly. After each addition the precipitate is allowed to settle somewhat and the bottom of the flask studied for the first trace of green precipitate, the end reaction, which will first settle here. Having determined how much of this solution will exactly precipitate the phosphoric acid of 50 cc. of solution No. 1 it is then diluted to approximately the proper amount and then again for a final exact correction. Twenty cc. of this solution indicate 0.1 gm. of  $\text{P}_2\text{O}_5$ .

For the estimation of phosphoric acid in the urine 50 cc. of urine are treated in exactly the above manner. If very accurate results are desired a table of corrections for the change in titer necessary for the volume used should be at hand to make the necessary changes. If the urine be colored or jaundiced, the end reaction will not be sharp, and it should be acidified with hydrochloric acid or nitric acid and decolorized with  $\text{KMnO}_4$ . The urine should then again be neutralized. During the titration the flask should be kept on the water-bath or over the free flame to keep the fluid almost at the boiling point. Between each addition the precipitate should be allowed to settle that the first trace of green may be seen; the longer it is allowed to settle the sharper the end reaction.

Instead of the cochineal a 10 per cent. solution of potassium ferrocyanide solution may be used. In this case, after each addition of uranium nitrate one drop of the hot solution is brought into contact on a porcelain plate with one drop of this reagent. The end reaction is a brown precipitate. It should be remembered that this is not the same end reaction obtained by cochineal, but one considerably later, hence in using the fluids it is essential that one know with what indicator it was standardized.

The determination is very satisfactory, and a class all using the same urine get very close results.

**DETERMINATION OF THE ACIDITY OF THE URINE.**—The acidity of the urine is so much due to the presence of diacid phosphates that for many years the determination of these salts was the most accurate way of determining the acidity.

Ereund's method is based on the fact that  $\text{BaCl}_2$  will precipitate the monacid, but in the dilute urine not the diacid salt. If, therefore, the total phosphoric acid

and the monacid salt be determined, the difference will be diacid phosphate. The method is not exact; the monacid-salt figure will be 3 per cent. too great. For the determination the above solutions are used; also one of 100 gms. of  $\text{BaCl}_2 \cdot 2\text{H}_2\text{O}$  to 1 litre of water. The total phosphoric acid in 50 cc. of urine is determined. To 75 cc. of urine the barium chloride solution is added till the whole is 90 cc. The fluid is then shaken well and filtered until clear. This may mean repeated attempts; 60 cc. of the filtrate (equalling 50 cc. of urine) are then used, the phosphoric acid determined and subtracted from the total. From the monacid phosphate is subtracted 3 per cent., which is added to the diacid. The acidity of the urine is expressed in terms of the diacid salts.

**Sulphates.**—Sulphur is present in the urine in three forms,—(a) preformed or neutral sulphates; (b) ethereal or conjugated sulphates, that is, sulphuric acid combined with aromatic alcohols, indoxyl, skatoxyl, cresol, phenol, *et al.*; (c) neutral, unoxidized, or organic sulphur. Weighed as  $\text{SO}_3$ ,  $a + b$  amount to from 1.5 to 3 gms., or an average of 2.5 gms. of  $\text{H}_2\text{SO}_4$  in twenty-four hours, in the case of a normal person on a mixed diet. As a rule, the ethereal sulphates are about one-tenth of the total sulphates. Since practically all of the sulphuric acid is a product of proteid metabolism, the output should be parallel to that of nitrogen, and the ratio between them is quite constantly 5:1 to 5 (100:19.1 to 20.4, Folin). But this is not exact, since the sulphur contained in proteids varies, and the amount of sulphur excreted in the neutral form varies as well.

The total sulphate output depends especially upon proteid metabolism, being increased in all conditions increasing proteid oxidization; hence there is none in the urine of the foetus. It is especially dependent on a meat diet. It is increased by exercise, providing this increases the nitrogen output as well. It is increased in fevers, since in this condition there is an increased proteid catabolism. The increase is especially marked in acute inflammatory disease of the brain and cord, and in acute articular rheumatism. It is increased after protoplasmic poisons. It is diminished during convalescence from an acute fever and in practically all chronic diseases. The amount of total sulphates has very little clinical value.

The **ethereal sulphates** are of considerable interest. While their output is subject to great and inexplicable variations, they may be considered as an accurate index of the amount of absorption of the products of intestinal decomposition which can pair with the acid. They are independent in great degree of the neutral sulphates, and with the total sulphate their ratio varies so considerably that normal limits cannot be stated, hence it is their absolute amount which is of more value than their relative.

This amount varies in the first place with the food. They are increased in the urine of a dog fed on foul meat; are diminished during hunger and long fasting; they are diminished on a milk diet, in which case it is supposed that casein inhibits the bacteria of decomposition.

Intestinal decomposition increases them relatively and absolutely. This increase is diminished much by calomel and similar drugs. The decomposition of proteid furnishes phenol, cresol, indoxyl, skatoxyl, hydrochinin, pyrocatechin, and several other bodies; the most important, indol and phenol, explaining only about one-fifth, and a large percentage is still unidentified. They are increased by the ingestion of aromatic bodies, and particularly of carbolic acid. There is almost none in the urine of newborn. The output varies to a great extent with the hydrochloric acid of the gastric juice and the sodium chloride of the food. After hydrochloric acid medication they are diminished, and are increased by alkaline drugs.

Pathologically, they are increased in chronic intestinal catarrh and diminished in acute. They are increased sometimes in constipation, sometimes there is no change. In a recent interesting case of "black urine" (see page 106), from a case of extreme constipation, the total sulphuric acid (as  $\text{SO}_3$ ) was only 0.147 gm. per 100 cc. of urine, and of this, 57 per cent. was ethereal sulphate; the following day the urine was of normal color, total  $\text{SO}_3$ , 0.086 gm. per 100 cc., and 50 per cent. of this ethereal sulphate. They are increased in those cases with defective absorption from the intestine, as in typhoid fever, intestinal tuberculosis, and peritonitis. They are increased in cholera, but during the stage of reaction there may be little or none present. In atrophic liver cirrhosis and carcinoma of the liver the increase is attributed to the accompanying intestinal catarrh. They are also increased if decomposition occurs in other parts of the body than the intestine. It is of interest that in gastric disease, even with much stagnation and fermentation, they are little affected.

The **unoxidized sulphur** is supposed by some to vary with the amount and quality of the food; by others to have relation not to food but to tissue destruction, to be increased by muscular work, the lack of oxygen, and the ingestion of various sulphur compounds, including the flower of sulphur, sulphonal, methylmerkaptan and ethyl sulphide.

This neutral sulphur, which amounts to from 14 to 25 per cent. of the total sulphur, is present in two forms: about 20 per cent. is the easily oxidizable, which is oxidized by bromine or chlorine (bromine is better, since chlorine attacks also the taurin derivatives), and the difficultly oxidizable. For the total the residue must be fused with  $\text{KNO}_3$ , since fuming  $\text{HNO}_3$  does not oxidize all of the neutral sulphur. In cystinuria  $\text{HCl}$  plus  $\text{KNO}_3$  will oxidize only from 30 to 40 per cent.

In jaundice from 24 to 60 per cent. of the sulphur is neutral, and of this there is an increase of about four to five times the normal proportion of the difficultly oxidizable form. In pneumonia the increase is of the easily oxidizable, and in liver disease the opposite. In cystinuria even 45.7 per cent. is neutral sulphur (in one of our cases 32 per cent.).



Edsall<sup>39</sup> carefully studied the easily split (by alkali) sulphur in a series of cases. His results were negative. He decided that cystinuria is the only disease with an increase in this sulphur fraction, and that the relative proportion of these two fractions has no clinical value.

Petry studied the question in dogs on a known diet, and found a quite constant amount (5.5 per cent.) of the total sulphur to be the easily split. This amount could not at all be influenced by diet.

Recent work by several (*e.g.*, Benedikt)<sup>40</sup> has emphasized the independence between the excretion of neutral and total sulphur, the former remaining almost constant whatever the diet. It is suggested that the neutral sulphur arises in the catabolism of particular proteids.

**DETECTION AND APPROXIMATE ESTIMATION.**—If in a test-tube holding over 25 cc. the urine is mixed with about one-third its volume of an acid barium chloride solution ( $\text{BaCl}_2$ , 4;  $\text{HCl}$ , 1;  $\text{H}_2\text{O}$ , 16 parts), a precipitate of the barium sulphate is formed. If this be a milky turbidity, the sulphates are normal; if creamy, increased; if merely a translucency, diminished. If allowed to settle from eighteen to twenty-four hours, and the precipitate fills one-half the concavity of the tube, they are normal. The above are the neutral sulphates. If this be filtered, and to the filtrate hydrochloric acid be added and the whole warmed, the ethereal sulphates are split and precipitated.

**QUANTITATIVE DETERMINATIONS OF SULPHUR-CONTAINING BODIES.**—The determinations of the sulphur bodies of the urine, both the neutral and ethereal sulphates and the total sulphur, are so important in metabolism experiments that the student should be trained in the general methods of this work. He should remember that these determinations, theoretically so easy and accurate, are in reality very difficult and so full of pitfalls that much preliminary practice should precede any important work. In the former editions of this book we have in general followed the directions given by Huppert and Salkowski, but recently the careful work of Polin\* has appeared, and his directions are the result of so careful study and are given in such detail that we have copied them in full. The laboratory worker is advised, however, before beginning work on the sulphur bodies to read the whole of the excellent paper of which only portions are copied here.

**INORGANIC SULPHATES.**—About 100 c.c. of water (not less), 10 c.c. of dilute hydrochloric acid (1 part concentrated  $\text{HCl}$  to 4 parts  $\text{H}_2\text{O}$  by volume), and 25 c.c. of urine are measured into an Erlenmeyer flask (capacity 200–250 c.c.). If the urine is dilute, 50 c.c. instead of 25, and a correspondingly smaller quantity of water

<sup>39</sup> Univ. of Penn. Med. Bull., 1892, iii, p. 87.

<sup>40</sup> Zeitschr. f. klin. Med., 1899, vol. xxxvi, p. 281.

\* On Sulphate and Sulphur Determinations, The Jour. of Biol. Chem., Jan., 1906, vol. i, p. 131.



may be taken. A five per cent. solution of barium chloride solution (10 c.c.) is then added, always drop by drop, preferably by means of an automatic dropper. The urine solution is not to be shaken, stirred, or otherwise disturbed while the barium chloride is being added. At the end of an hour or later, according to convenience, the mixture is shaken up and filtered through a Gooch crucible. The precipitate is washed with about 250 c.c. of cold water, dried, and ignited.

Filter papers (S. & S. blue-ribbon filters) may be used, but Folin found that series of parallel determinations made with these showed greater variations and a lower total average than the series made with the Gooch crucible. He advises that the porcelain crucibles rather than the platinum be used and describes the following technic:

The asbestos for the mats must be good material, consisting chiefly of long shiny fibres. The fibres are cut with scissors into suitable lengths (50–70 mm.). A few grams at a time are then placed in a cylinder with about 300 c.c. of 5 per cent. hydrochloric acid, and a strong air current is passed through for a few minutes. This separates all the fibres far more quickly and completely than the usual method of scraping them with a knife. In an hour or two asbestos enough for two hundred crucibles can be prepared. It is kept ready for use in dilute hydrochloric acid. From 50 to 100 mgs. of asbestos are used for each mat. By using a good vacuum pump at almost full force the asbestos mat is packed into a thin but uniform and firm layer in the bottom of the crucible. It is then washed with the help of only enough of a vacuum to make the water run through in a slow stream; it is finally dried, ignited, and weighed. Mats so prepared are as effective as the best filter paper in retaining precipitates, and there is practically no danger of losing any asbestos during the subsequent washings of precipitates of barium sulphate. The same mat can advantageously be used until about 1 gram of barium sulphate has collected. Time is saved by not using the same mat too long, because the filtration becomes slower and slower, the more precipitate there is present, and it is not safe to increase the vacuum too much.

The ignition of the precipitates is associated with more serious sources of error than the filtration, more serious because they are not accessible to direct observation. The flame must not be applied directly to the perforated bottom of the crucibles. If this is done mechanical losses are sure to occur, even though the crucibles are covered with lids. Nor is it safe to apply the flame to the sides of the crucibles. To do so involves again mechanical loss of barium sulphate. During the ignition the crucibles must be provided not only with lids, but also with tight bottoms. This is easily accomplished by the use of lids of ordinary platinum crucibles. The lid is placed on a triangle, and the crucible stands in upright position on top, while

the flame is applied to the platinum lid. These points may seem trivial, but they consume no extra time, and they are necessary for uniformly reliable figures. Ten minutes' ignition is sufficient, unless organic matter is present.

**TOTAL SULPHATES (NEUTRAL [*i.e.*, INORGANIC] AND ETHEREAL).**  
—Of the following two methods Folin prefers the first: *a. Barium Sulphate Precipitation in the Cold.*—Twenty-five c.c. of urine and 20 c.c. of dilute hydrochloric acid (1 part HCl, sp. gr. 1.20, to 4 parts H<sub>2</sub>O), or 50 c.c. of urine and 4 c.c. of concentrated hydrochloric acid, are gently boiled in an Erlenmeyer flask (capacity 200–250 c.c.) for 20 to 30 minutes (not less than 20). To reduce the loss of steam it is better to keep the flask covered with a small watch glass during the boiling. The flask is cooled for two or three minutes in running water, and the contents are diluted with cold water to about 150 c.c. To this solution is then added 5 per cent. barium chloride (10 c.c.) without any shaking or stirring during the addition. The remainder of the procedure is like that of the inorganic sulphate determination.

*b. Barium Sulphate Precipitation in the Heat.*—The boiling of the urine with hydrochloric acid is conducted exactly as in the preceding method. At the end of 20 to 30 minutes the boiling urine is diluted to about 150 c.c. with hot water. The mixture is heated once more to the boiling point, is then taken off the fire, and at once precipitated with ten per cent. barium chloride solution (5 c.c.). The barium chloride must always be added drop by drop. The filtration is made after about two hours' standing, when the mixture has acquired the room temperature. The remainder of the procedure is like that of the determination of inorganic sulphates.

*Ethereal Sulphates.*—There is no need that these be separately determined, since the difference between the amounts of total and neutral sulphates found in any given specimen will be an accurate index of the ethereal sulphate in that particular specimen.

*Total Sulphur.*—The determination of the total sulphur is one of the most important but most difficult problems of proteid metabolism. Folin's method is the following:

Twenty-five c.c. of urine (or 50 c.c. if very dilute) are measured into a large nickel crucible (capacity 200–250 c.c.), and about 3 grams of sodium peroxide are added. The mixture is evaporated to a syrupy consistency, and is then carefully heated until it solidifies. This heating may seem a little slow, requiring about fifteen minutes, but the conditions have purposely been selected to make it slow (by using as much as 3 grams of Na<sub>2</sub>O<sub>2</sub>), in order to drive off as much ammonia as possible before the final fusion with more peroxide. The crucible is removed from the flame and allowed to cool. The residue is then moistened with 1 or 2 c.c. of water, and, after about 7

grams of sodium peroxide are sprinkled over the contents in the crucible, the mixture is heated to complete fusion for about ten minutes.\* After cooling for a few minutes, water is added to the contents in the crucible, and the mixture is heated for at least half an hour with about 100 c.c. of water to dissolve the alkali and to decompose the sodium peroxide. The mixture is next rinsed into an Erlenmeyer flask (capacity 400–450 c.c.) by means of hot water, and diluted to about 250 c.c. Concentrated hydrochloric acid is slowly added to the almost boiling solution until the nickelic oxide just dissolves (about 18 c.c. of acid to 8 grams of peroxide). After a few minutes' boiling the solution should be perfectly clear. If it is not clear too much water or too little peroxide has been added for the final fusion. The insoluble residue must then be removed by filtration (after cooling), because it will not dissolve on the addition of more hydrochloric acid, and too much acid must be avoided. The difficulty does not arise if little water and 7 or 8 grams of peroxide are used.

To the clear acid solution are added 5 c.c. of very dilute alcohol (1 part alcohol to 4 parts  $H_2O$ ), and the boiling is continued for a few minutes. The alcohol removes the last traces of chlorine, which is always formed on acidifying the solution. Ten per cent. barium chloride solution (10 c.c.) is next added (by means of a dropper), and the solution left standing in the cold for *two days* before filtering. The rest of the procedure is the same as for the other sulphate determinations.

**Thiosulphuric Acid,  $H_2S_2O_3$ .**—Normally there is in the urine none or not over 10 mg. in 1 litre, though it has been found in some cases, as in typhoid fever.

**Hydrogen Sulphide,  $H_2S$ .**—This seldom occurs in fresh urine. It has, however, been found, and by it have been explained cases of autointoxication. It was found in one case of long-standing eclamptic coma. It soon appears, however, in a urine on standing. It may be detected in the fresh urine by the odor, or by suspending in the mouth of the flask a strip of paper moistened with sugar of lead solution plus one drop of  $NaOH$ . Air should then be aspirated through the urine. The paper will be blackened.

**Sulphocyanic Acid,  $HSCN$ .**—This acid occurs normally in the urine of man and the animals which excrete nitrogen as urea, in amounts equalling about one-third of the neutral sulphur. To 100 c.c. of urine are added  $HNO_3$  and  $AgNO_3$ . The precipitate is filtered, washed, suspended in water, decomposed with  $H_2S$ , and the filtrate distilled. The distillate is tested with  $Fe_2Cl_6$ , giving an intense blue fluid (Berlin blue) not modified by  $HCl$ .

**Carbonates.**—Carbonic acid is present in the urine, both free, which may be removed by a vacuum, and bound, in which case acid must be added to free it. Of the free there are about 180 cc. of  $CO_2$ ; of the bound, from 2 to 10 cc. The carbonic acid is increased by a diet rich in organic acids which are oxidized to carbonates, hence it is present in great abundance in the urine of herbivora.

**Silicic Acid** is present in traces as silicates. Its source is the food.

**Nitric Acid** is present in all normal urines as nitrates. This is from the food.

\* A little water should be added before the final fusion, to obtain a complete fusion by the aid of comparatively little heat. This protects the crucible against corrosion, almost completely preventing the presence of nickel in the final solution.

*Nitrous Acid* is often found as nitrites, but is reduced from the nitrates by the bacteria.

**Calcium and Magnesium.**—These alkaline earths are excreted as phosphates to the amount of about 1 gm. per day; calcium, weighed as CaO, about 0.12 to 0.25 gm., and MgO from 0.18 to 0.28 gm. per day. The calcium excretion, even of that injected subcutaneously, is chiefly through the intestines, and from but 4 to 29 per cent. through the urine. On this account the calcium in the urine is no index of the amount absorbed from the intestine. In the urine its output is parallel to that of the ammonia, and it seems to bear some relation to the excretion of acids. In this connection it is of interest that most is excreted in the morning, at which time the urine is most acid. Its chief source is the food. During starvation periods calcium is increased relatively and absolutely, which is of interest since there is also then a slight acidosis. The source of this calcium is assumed to be the bones. It can be decreased by alkaline treatment. On a vegetable diet there is only a trace of calcium in the urine. It seems increased by exercise.

The factors influencing the output of calcium are little understood, and yet much would indicate that the calcium bears some relation to the condition known as acidosis (see page 209). There is no increase in tuberculosis, and none in rickets. In chronic diseases the increase can be explained by inanition. In diabetes an interesting behavior of this metal was demonstrated by Gerhardt and Schlesinger, who by very careful metabolism work confirmed the previous findings that the output is in diabetes increased even two to four times the normal amount; that the output is parallel to that of ammonia and can be diminished by alkaline treatment; and that in those cases with acidosis it is especially increased; the normal ratio between the intestinal and the urinary output is reversed in favor of the latter, while there seems a certain amount of retention of magnesium in the body. In the case of arteriosclerosis a retention of calcium has been demonstrated.

The relation of this metal to phosphaturia is interesting, since that symptom-complex would seem to be due more to an increase of calcium with a diminution in the phosphoric acid than to an increase of the latter.

**QUANTITATIVE DETERMINATION OF CALCIUM.**—Two hundred cc. of filtered urine are made alkaline with ammonia until there is a distinct precipitate. This is then dissolved in the smallest amount of hydrochloric acid with the addition of some NaAc. Ammonium oxalate is then added in excess and the fluid allowed to stand covered on a water-bath for twelve hours. The precipitation of calcium phosphate which occurs if no ammonium be added or if the urine be foul should be avoided. The supernatant fluid is then decanted through a small ashless filter, the precipitate washed Cl free by decantation with hot water, and then finally brought on the paper. The precipitate of CaO is very fine and apt to pass through the paper,

hence is washed as much as possible by decantation. The wash-water may be saved for magnesium determination. During this process bacterial fermentation should be prevented by thymol or carbolic acid. The dry filter paper is then put in a platinum dish, burned moderately for a long time, then at a dull red, till the mass on cooling is perfectly white. It now contains some oxide. It is moistened with a concentrated solution of ammonium carbonate, slowly dried, and very gently ignited. The treatment with ammonium carbonate is repeated till of constant weight as calcium carbonate. One part of  $\text{CaCO}_3$  equals 0.40 parts of Ca. Or the mass may be burned white with a blast-flame. The crucible is then cooled and weighed and the blast repeated until the weight is constant. The precipitate is now  $\text{CaO}$ , 1 part equalling 1.845 of calcium phosphate. Or the precipitate is burned white, then concentrated ammonium sulphate added, again burned, and this repeated until there is no increase in weight. One part of this calcium sulphate equals 0.41176 part of  $\text{CaO}$ .

**QUANTITATIVE DETERMINATION OF MAGNESIUM.**—For this the filtrate and wash-water of the above determination may be used. One-third volume of 10 per cent.  $\text{NH}_4\text{OH}$  is added (sp. gr. 0.96), which will precipitate all of the Mg as  $\text{NH}_4\text{MgPO}_4$ . This is allowed to settle well, collected on an ashless filter, washed with water plus one-third volume of ammonia, thoroughly dried, shaken into a platinum crucible, the paper burned in a platinum spiral and its ash added to the crucible, and the whole then fused. Since there is some uric acid in the precipitate it will hardly burn white. It should therefore be cooled, a small piece of  $\text{NH}_4\text{NO}_3$  plus a few drops of water added, this warmed slowly, and then finally burned. The result is  $\text{Mg}_2\text{P}_2\text{O}_7$ . One hundred parts of this equal 36.208 parts of  $\text{MgO}$ .

It is a saving of time to treat 200 cc. of the original urine in this way. The result is calcium and magnesium. The Ca is determined in a second portion, and the difference will be the Mg.

**Sodium and Potassium** are present, of the former 4.2 to 7.4 gms. as  $\text{Na}_2\text{O}$  in twenty-four hours, and of the  $\text{K}_2\text{O}$  from 2.3 to 3.9 gms., the usual relation between them being 5 : 3.

Their amount depends on the food. In hunger the potassium may exceed the sodium, also in fever, but after the crisis the sodium will predominate.

Severe exercise and vegetable diet increase the potassium.

**Iron.**—A trace of iron is always present in the urine in organic combination. The figures given of the amount found vary widely, since all methods have many sources of error, in many cases the iron of the reagent used being greater in amount than that to be determined. The figures given vary from 1 to 10 mg. in twenty-four hours. This is increased in fever, the amount varying as the height and duration of the elevation of temperature. Large amounts have been found in malaria (even 16 mg. a day), pernicious anæmia, and alcoholics. This question has recently been studied by Neumann, who considers that his method of ashing the urine allows a very accurate estimation of the iron. Neumann and Mayer<sup>42</sup> found that the output of a normal person varied from 0.93 to 1.139 mg., an average of 0.983. In pathological urines they found it increased, especially in alcoholics. Their finding concerning diabetes is very interesting, since the iron output was parallel to that of the sugar; the ratio

<sup>42</sup> Zeitschr. f. physiol. Chem., 1902, vol. xxxvii. p. 2.

being quite constantly 2.5 mg. per 100 gms. of sugar. They therefore suspect that both bodies have a common source.

**Lead.**—Considerable urine is evaporated to dryness, and 50 cc. of fuming  $\text{HNO}_3$  added; after the reaction subsides it is allowed to simmer over the free flame for half an hour, and then 25 cc. more acid added three times, each fifteen minutes. The fluid is then evaporated to small volume, neutralized with  $\text{NaOH}$ , filtered, and the lead tested with  $\text{H}_2\text{S}$ , which will give a brown precipitate.

**Arsenic** may be tested by saturating the faintly acid urine with  $\text{H}_2\text{S}$ , allowing it to stand from twelve to twenty-four hours, filtering, washing, and treating the precipitate with bromine water, which will dissolve the arsenic sulphide. The solution is placed in a suitable flask, to which is added zinc and sulphuric acid, and the stream of hydrogen conducted into an acid  $\text{AgNO}_3$  solution ( $\text{AgNO}_3$ , 0.1 to 0.2 gm.;  $\text{HNO}_3$ , 2 gms.; water, 10 cc.). If  $\text{AsH}_3$  is generated, one gets a blackish-brown precipitate of metallic arsenic.

#### PIGMENTS OF THE URINE

The value of the ethereal compounds of sulphuric, glycuronic, and other acids has been overestimated, yet they have a certain clinical importance.

**Indoxyl sulphate**, the chief body, originates in the intestine as indol which is formed in the decomposition of proteid. Indol is absorbed from the intestine, and in the body is oxidized to indoxyl, conjugated with sulphuric acid, and excreted as an alkaline salt. In men its output is greater on a flesh than on a vegetable diet. In the case of fasting persons it arises from the decomposition of the intestinal secretions. There is none in the urine of the new-born or until the child is fed cow's milk. In adults a certain amount is always present, from 5 to 25 mg. in twenty-four hours on a mixed diet; hence only a great increase is of value, and this may reach from 50 to 150 mg.

In general, it is increased by the rapid decomposition of albumin either in the intestine or elsewhere in the body. The increase in cases with limited peristalsis—*e.g.*, peritonitis and ileus—is of importance only to indicate the location of the obstruction or of the paralysis of the bowel. If the obstruction is in the small intestine, there is a great and rapid increase; if in the colon, either there is no increase or one beginning late. Its formation seems to depend upon the presence of trypsin, which before it reaches the colon has been either destroyed or reabsorbed. It cannot be used to distinguish peritonitis from a twist of the bowel. In one very interesting case of syphilitic stricture of the ileum the obstruction, which occurred frequently, due evidently to the accumulation of fecal matter above the constriction, could be

foretold by the increase of this body, which reached a high point and then, as the food gradually passed on, cleared up much. It is much increased in intussusception, new growths, and twists of the small intestine. It is increased by intestinal putrefaction, such as occurs in diarrhœa, especially the cholera infantum of children, in typhoid fever, dilated stomach, and some cases of nephritis. In these conditions brisk purging diminishes the output greatly.

It is increased by decomposition of albumin elsewhere in the body, as, for instance, in gangrene of the lung tissue, gangrenous empyema, putrid bronchitis, in which case it may be very large in amount, advanced pulmonary tuberculosis, and advanced intestinal tuberculosis. Coriat considers its increase one element of the symptom-complex of akinetic mental conditions, and its diminution one element of that of the hyperkinetic states. He considers this not due to any intestinal condition nor to the diet. In certain cases of chronic constipation it is present in large quantities. One such case, a colleague of mine, furnished my classes for several years with specimens of urine rich in this pigment. This person enjoyed the best of health. He gives a history of some severe abdominal condition when ten years of age, since which time he has been troubled with constipation.

His urine on one day was as follows:

Total amount, 1770 cc, clear yellow color. On boiling, it becomes dark brownish-red, almost black, with a dark magenta foam.

Total  $\text{SO}_3$ , 1.59 gms.; ethereal sulphates, only 14 per cent.! Total sulphur, 1.82 gms. (as  $\text{SO}_3$ ) in twenty-four hours.

The urine gives a splendid indigo-blue test, not the Rosenbach test.

It will be seen that despite the color on boiling and the good indoxyl test, the ethereal sulphates were not increased.

The output is diminished by closure of the pancreatic duct, but this closure cannot be diagnosed from the absence of indoxyl sulphate unless other factors which would favor its formation are present. A former idea was that it is increased in conditions of inanition and tuberculosis, and there is a long list of diseases in which it has been found in abundance, namely, various intestinal troubles, cancers of the liver, stomach, or uterus, and lead colic. In cases of peritonitis or of appendicitis with abscess, an increase of indican is an unfavorable sign; its decrease, a favorable one. It is increased when the HCl of the gastric juice is diminished. It seems to bear some relation to the albumin output, and the insurance companies are beginning to consider it an early feature of nephritis. This opinion, however, needs confirmation.

Phenol is almost always increased with it; the reverse, however, is not true.

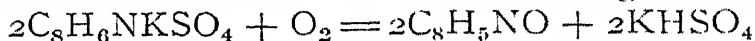
The urine when voided is, as a rule, normal in color. In certain cases the oxidation occurs within the body and the urine is green



when voided (the blue of the indigo plus the yellow of the urine). Such cases are described by Sahli, MacPhedran and Goldie, and others, but are excessively rare, and methylene blue is always to be excluded (see page 106).

Indigo calculi have been found.

The demonstration of indoxyl depends on its oxidation to indigo-blue, and the index of amount is the amount of indigo thus formed.



This reaction occurs if an oxidizing agent be added; also when the urine decomposes, in which case it is present as a copper-red scum with a metallic glistening. Rarely, however, is there sufficient to be seen grossly.

Indigo-blue is a dark blue powder, insoluble in water, slightly so in chloroform, easily soluble in hot aniline. It is insoluble in alcohol and ether. It should be collected on an asbestos filter, washed with water, then with alcohol (to separate the indigo-red), and then dried.

Tests: It may be sublimed at  $300^\circ\text{C}$ , giving off a purple-red vapor which cools in prismatic crystals of a copper-red metallic color, deep blue on transmitted light. If indigo-blue be mixed with hot alcohol, very strong NaOH, and some glucose, the whole filling a closed flask, indigo-white is formed. If then exposed to the air, indigo-blue will recrystallize out.

**TESTS OF INDOXYL SULPHATE.** *Jaffé's Test.*—A test-tube is half filled with urine, another of the same size with the same amount of concentrated HCl. On the edge of this latter test-tube is placed the smallest drop of fresh concentrated  $\text{Ca}(\text{ClO})_2$ . The HCl is then poured quickly into the urine, carrying with it this drop as it flows over the edge. The fluids are mixed rapidly by inverting, not by shaking the tube. One cubic centimetre or so of chloroform is then added, which will extract the indigo as it is formed. If necessary then another drop or two of the  $\text{Ca}(\text{ClO})_2$  solution may be added. The test may be performed as a contact test. Hammersten advises that to 20 cc. of urine 2 or 3 cc. of chloroform be added, and an equal amount of hydrochloric acid; then at once  $\text{Ca}(\text{ClO})_2$  drop by drop, reversing the tube several times after each addition. The difficulty with this test is that a slight excess of the hypochlorite will destroy the indigo, giving yellow isatin. Albumin must be first removed by boiling and filtering.

$\text{Ca}(\text{ClO})_2$  is a difficult substance to obtain pure, since it deteriorates so rapidly that the manufacturing chemists refuse to import it. A pure salt, however, is unnecessary, the ordinary cheap bleaching powder or "chloride of lime" being satisfactory. (Chloride of lime, it should be remembered, is not calcium chloride, but a mixture of calcium hydroxide, calcium chloride, and calcium hypochlorite).

*Obermayer's Test.*—The urine is first precipitated with about one-fifth volume of 20 per cent. PbAc, avoiding an excess, then filtered. Disturbing substances are thus removed. An equal amount of fuming



hydrochloric acid containing  $\text{Fe}_2\text{Cl}_6$  is then added (4 parts of  $\text{Fe}_2\text{Cl}_6$  in 1 litre of  $\text{HCl}$ ). In a few minutes the reaction is apparent, and the indigo can be extracted with chloroform.

If potassium iodide is in the urine, a violet color is obtained. With thymol the urine becomes bluish-green.

*Aman's* reaction with  $\text{Na}_2\text{S}_2\text{O}_7$  has been found of doubtful value.

The test may also be performed by adding 30 drops of urine to 15 cc. of  $\text{HCl}$ , plus 1 or 2 drops of  $\text{HNO}_3$ . The mixture is stirred at once, and an amethyst color results, which reaches a maximum in from five to thirty minutes.

Since nitric acid gives the test, indol may disturb a bile test. In case the urine looks grossly as if indigo were present (the color of such a urine will be blackish-green or bluish), the indigo may be extracted with acidulated chloroform.

Indigo will crystallize out in needles or plates if evaporated from chloroform.

**QUANTITATIVE DETERMINATIONS.**—These are in general unsatisfactory, the gross estimate by the color, using the Obermayer reagent, being usually sufficient. This urine may be repeatedly extracted, the extracts evaporated in a weighed beaker. The indigo-red may in this case be removed by washing with alcohol. It is dried at  $105^\circ$  to  $110^\circ$  C. and weighed.

Ellinger<sup>43</sup> found that but 85 per cent. of the theoretical amount was in this way obtained (evidently isatin is formed), and that neither the concentration nor the excess of reagent was of moment if one quickly extracted the indigo-blue. The urine is, if necessary, made slightly acid with acetic acid, precipitated with one-tenth volume of  $\text{PbAc}$  and, if concentrated, diluted one-half. To a measured portion of the filtrate is then added an equal volume of Obermayer's reagent, and shaken out several times with chloroform until this is no longer colored. The amount of filtrate chosen should be such that three to four extractions with 30 cc. of chloroform, for two minutes each time, is enough. The filtered extract is distilled, the extract dried for five minutes, then washed out two to three times with hot water (to remove isatin), dissolved in 10 cc. of concentrated  $\text{H}_2\text{SO}_4$ , this solution diluted to 100 cc. with water, and titrated with a dilute  $\text{KMnO}_4$  solution (5 cc. of a 0.3 per cent. solution diluted to 200 cc.) which has been standardized with pure indigo-blue. About 87 per cent. of the correct amount is found, hence the result may be increased by one-sixth. A double determination requires about one and a half hours.

Strauss<sup>44</sup> also uses Obermayer's solution and extracts with chloroform. He uses a small separating funnel similar to that for lactic acid (see Fig. 63). The combined chloroform extracts are measured, 2 cc. removed and diluted till its color matches that of a standard tube of known content, and from this he reckons the total amount.

Bouma's method<sup>45</sup> has received severe criticism.

Coriat proposes<sup>46</sup> a graduated separating funnel, in which the  $\text{Ca}(\text{ClO})_2$  test is made and the chloroform extract compared with a standard color.

**SKATOXYL-SULPHATE.**—Skatol is also formed in the intestine, the result of the bacterial decomposition of albumin, and is absorbed. By analogy we may suppose it to be oxidized to skatoxyl, conjugated with sulphuric acid, and eliminated by the urine. As a matter of fact, however, the skatoxyl sulphuric acid has seldom, if ever, been actually demonstrated in the urine, and the colors which would indicate it may be as well due to other red pigments.

<sup>43</sup> Zeitschr. f. physiol. Chem., vol. xxxviii. p. 178.

<sup>44</sup> Deutsche med. Wochenschr., April 17, 1902.

<sup>45</sup> Zeitschr. f. physiol. Chem., 1901, vol. xxxii. p. 82.

<sup>46</sup> Am. Jour. Med. Sci., April, 1902.

Stokvis<sup>47</sup> has given a method for the separation of the chromogens of these blue and red pigments. The urine is saturated with  $(\text{NH}_4)_2\text{SO}_4$ , allowed to stand till all pigments are precipitated, filtered, the filtrate evaporated on the water-bath, and the fluid decanted from the crystals of  $(\text{NH}_4)_2\text{SO}_4$ . It is then acidified with a few drops of acetic acid, and shaken out with an equal volume of acetic ether, which takes up both chromogens to a yellow solution. To the acetic ether is now added water several times to separate out the chromogen of indigo-blue. It is then neutralized with dilute KOH and shaken out, the dilute KOH separating the chromogen of skatol-red, which can be tested with Obermayer's solution.

The red or violet color produced by adding an oxidizing agent with a strong acid to the urine is usually attributed to this body. With  $\text{Fe}_2\text{Cl}_6$  it does give a violet color, with concentrated  $\text{HNO}_3$  a cherry-red color, with concentrated  $\text{HCl}$  it is decomposed with a red precipitate. In Jaffé's test the urine is dark red or violet. On standing in the air the urine becomes darker from above downward,—of a red, violet, or even black color. As has already been mentioned, these colors are not conclusive. Rosin denies that it has ever been found, and thinks all of these tests could be explained by indigo-red. Its demonstration would necessitate the reduction of the pigment by zinc-dust with skatol as the product.

**Indigo-red.**—Other names for this pigment are many, uro-rubin and uro-rhodin being among them. This body is always formed with indigo-blue, especially by Jaffé's test with the warm urine. They are isomeres and arise from the same mother substance (indoxyl sulphate). It is also formed in decomposing urine and may form a sediment. Uro-rosein is formed at the same time.

Indigo-red crystallizes in dark reddish-brown or chocolate-brown needles or plates. It sublimates with violet-red fumes at  $295^\circ$  to  $310^\circ$  C. It is insoluble in water, dilute acids, and alkalies. It gives a cherry-red solution with alcohol, ether, chloroform, and especially glacial acetic acid. From dilute alcohol solution it precipitates in crystals. From glacial acetic acid it is precipitated by soda or by water. It gives a characteristic absorption spectrum.

**REDUCTION TEST.**—The alcohol solution is made alkaline with sodium carbonate, a little glucose added, and gently warmed. The solution is decolorized, but the color returns on shaking it in the air. This can be repeated as often as desired. If the pigment be boiled with caustic alkali, even dilute, the red is destroyed and various brown decomposition products formed.

This pigment is present in large amounts in certain urines which give Rosenbach's test, but it alone is not responsible for the Burgundy-red color. It is increased especially in intestinal troubles,—ileus, obstruction, cancer, etc. It is also present in large amounts in some urines which do not give a characteristic Rosenbach test, but these conditions are so various that they cannot be classified. It may also be found in traces in normal urine.

**DEMONSTRATION.**—Nitric acid is the best reagent, its addition giving a red color. Much is formed by Jaffé's test, especially if the urine be heated. When cold, the urine may be neutralized with soda, then shaken out with ether. The ether takes a fine red color and gives the absorption spectrum of this body. The ether extract may be evaporated in a watch-glass to obtain crystals.

Indigo-red is present in certain freshly voided urines as in cases of pyelocystitis. It has also been found in concretions.

<sup>47</sup> Centr. f. inn. Med., 1902, No. 28.

Among other red pigments of the urine is **UROROSEIN**. This is characterized by its easy solubility in amyl alcohol, its insolubility in chloroform, ether, and benzol. Ammonia and alkaline carbonates decolorize it at once; acid restores the color. It may be recognized from its spectrum. It is very unstable and decomposes rapidly. It occurs in normal urine.

To demonstrate it one-tenth volume of HCl is added, and then the fluid is filtered. The red stain which remains on the filter is urorosein. Urorosein is produced by Jaffé's test, but is not extracted by the chloroform or ether. Other red pigments may be demonstrated in the urine, and after boiling it with acid various brown pigments with which it is tempting to work but not very profitable. These pigments, somewhat similar in appearance but different in their solubilities, will puzzle one considerably.

**Paracresol and Phenolsulphuric Acid.**—Phenol is present in the urine, from 17 to 51 mg. per twenty-four hours. The amount of these two bodies, however, of which the former usually exceeds in amount, varies in various conditions. It is increased with a vegetable diet. In ileus and peritonitis much phenol is present, also in diphtheria, scarlet fever, erysipelas; but little in typhoid fever, smallpox, and meningitis. They are formed from decomposition in any part of the body and when the intestinal decomposition is much increased. Their occurrence is thus much the same as indoxylsulphuric acid, the phenol increasing with this, but the reverse is not always the case.

**Pyrocatechin** (see page 105) is another body conjugated with sulphuric acid, and

**Hydrochinon** (see page 105) also, especially after carbolic acid poisoning.

**Potassium Iodide** is often found in the urine when making the tests for pigments. If  $\text{HNO}_3$  be added, and then chloroform, the latter will take the pink color of iodine. Or powdered starch may be added after the  $\text{HNO}_3$ , and the starch iodine blue will be very distinct.

#### BILE PIGMENTS

**The Clinical Occurrence of Bile Pigments in the Urine.**—Bile pigment never occurs in the human urine normally, although it is a constituent of the normal urine of some animals. Its origin is the blood pigment, as may be seen by the fact that it is increased and the intermediate stages occur in the plasma, when there is increased breaking down of red blood-cells,—*e.g.*, in hæmoglobinæmia, after a blood poison. It is very similar to hæmatin, and is an isomer of hæmatoporphyrin. In cholæmia it is thought that the most of the bilirubin is reduced by the kidneys to the more diffusible urobilin.

The cases of jaundice have been divided into two groups,—the “hepatogenous,” due to obstruction of bile passages, in which case bile

is present in the urine sooner or later, as in cases of catarrhal jaundice, jaundice due to calculus, cancer or cirrhosis of the liver; and the "hæmatogenous," formerly supposed to be due to destruction of the red blood-corpuscles by poisons, as phosphorus or the toxins of severe infections, in which cases bile may appear in the urine before the skin or conjunctivæ are stained. The previous idea was that in these latter cases the liver could not warehouse all of the free hæmoglobin, and hence some was excreted as bilirubin. More recently such jaundice has been doubted (Stadelmann), and all cases are supposed to be hepatogenous in origin; that is, the increased bile pigment is reabsorbed in the liver, since it is perhaps too viscid to flow well through the bile ducts, or there is too little pressure from behind, or perhaps there is closure of the smallest ducts from swelling of the cells. "Toxæmic jaundice" has been proposed as a better term.

The bile pigments, and their derivatives of interest in clinical chemistry, are bilirubin, biliverdin, bilifuscin, biliprasin, cholecyanin, and choletelin, all of which are products of bilirubin, and many of which are seen in the play of colors of the Gmelin test. The first two, bilirubin and biliverdin, are the only ones of much importance except in explaining the colors in tests. Bilirubin alone has been proved in fresh urine. Biliverdin often occurs, but, as a rule, only after the urine has stood for even a short time, in which case it is the effect of oxidization by bacterial action. These pigments are always present in the urine in cases of jaundice of much intensity. It should be remembered, however, that all of the pigment may be in the urate sediment.

**Bilirubin,  $C_{32}H_{36}N_4O_6$ .**—This is the pigment which occurs free in the bile of man. Its calcium salts occur in gall-stones, its crystals occur in old blood extravasations, the so-called hæmatoidin crystals supposed formerly to be a different substance but now generally admitted to be the same. It is probable that bilirubin can arise elsewhere than in the liver. It occurs in the fluid of certain cysts, especially of the breast and of the thyroid.

This pigment may be isolated from fluids containing other pigments by precipitating with the milk of lime in moderate amount, shaking well.  $CO_2$  is led in at once to prevent the decomposition of methæmoglobin et al., and the mixture filtered. The precipitate is washed, and dissolved in alcohol; chloroform is then added, and then acetic acid, to separate out the calcium. This is filtered, the chloroform separated by adding water, and the chloroform extract is filtered through a dry paper and evaporated. The bilirubin of the residue is washed with a little alcohol and ether. The results by this method are always a little too low, since calcium does not precipitate all the pigment and some is later destroyed. The work must be done rapidly. Hæmatin, if present, would also be isolated, but hæmatin does not occur in the body, except perhaps in the stomach and intestinal contents.

The crystals are rhombs often with rounded edges, or needles, if pure of a beautiful brown-red color. It is perfectly insoluble in water,

is soluble in alcohol and in chloroform, especially if hot, these solutions having a brownish-red color. It is precipitated unchanged from alcoholic solution by acids, forms compounds with alkalis, these compounds being insoluble in chloroform but soluble in water; hence bilirubin may be washed from a chloroform solution by an alkali. In this it differs from lutein. It is precipitated by  $\text{BaSO}_4$  and  $(\text{NH}_4)_2\text{SO}_4$ . The alkaline solution exposed to the air turns to the green biliverdin. In the alkaline urine, however, this is not always the case, since one product of the decomposition of urine is  $(\text{NH}_4)_2\text{S}$ , which changes biliverdin and bilicyanin to bilirubin, and the bilirubin itself may disappear from an alkaline urine, also from a urine preserved with chloroform. Bilirubin has no absorption spectrum.

**Biliverdin**,  $\text{C}_{32}\text{H}_{36}\text{N}_4\text{O}_8$ , occurs in the bile of many animals, but not of men. It occurs, however, in the intestine and vomitus, and in jaundiced urine after standing even for a short time. When pure it is an amorphous, greenish-black powder, insoluble in water, ether, or chloroform, but easily soluble in alcohol. Its compounds with the alkalis are soluble to a green or a brownish-green solution. It is soluble in concentrated acetic acid and in  $\text{HCl}$ . The alkaline solution has no absorption spectrum, but an alcoholic weakly acid solution shows one band. With Gmelin's test it gives the same color changes as bilirubin, from which it differs in its color, solubility in alcohol, and insolubility in chloroform. It may be reduced to bilirubin.

**Hydrobilirubin**,  $\text{C}_{32}\text{H}_{46}\text{N}_2\text{O}_7$ , is considered by some to be the same as urobilin, but this is denied by the majority. This occurs in the lower intestine as a reduced product of bilirubin by bacteria.

**Bilifuscin** is a body not yet isolated pure. That described ( $\text{C}_{32}\text{H}_{40}\text{N}_4\text{O}_8$ ) is of an amorphous brown color, soluble in alcohol to a deep brown solution, and in alkali, ammonia, and dilute  $\text{NaOH}$ . It is insoluble in water and ether, and nearly soluble in chloroform. It is soluble in ether and chloroform if fatty acids be present. In the pure state it does not give Gmelin reaction; its spectrum is similar to that of biliprasin.

**Biliprasin** is commonly said to be a mixture of bilirubin and bilifuscin. By others it is said to be an intermediate stage between bilirubin and biliverdin. Others consider it identical with biliverdin. The formula given is  $\text{C}_{32}\text{H}_{44}\text{N}_4\text{O}_{12}$ . The alcoholic solution has no absorption spectrum, while the alkaline solution has. The color of its alcoholic alkaline solution is brown, the chief difference between this and biliverdin. It differs from bilifuscin, since if acid be added to this solution, the color changes to green. The Gmelin test is of no value in recognizing this pigment.

Of the above pigments, the spectrum analysis is unsatisfactory for their recognition. It is the products of their oxidation which are easily recognized by this method.

**Cholecyanin**.—This is an oxidized product of the bile pigments with nitric acid,  $\text{PbO}$ , or  $\text{KMnO}_4$ . It gives a characteristic spectrum, and may be further oxidized to choletelin. Cholecyanin is insoluble in  $\text{H}_2\text{O}$ , soluble in alkalis and strong acids. It may be reduced to bilirubin. The neutral or faintly acid solution is of a bluish-green or steel-blue color with a beautiful red fluorescence. The

alkaline solution is of a green color. The only way of recognizing it is by its beautiful spectrum.

**Choletelin.**—Choletelin may be produced by the oxidation of bilirubin with  $\text{HNO}_3$ . It is soluble in alcohol giving a ruby-red color. The dilute solution is a yellowish-red, and does not change in color with the change of reaction, as is the case of urobilin. It does not fluoresce. Chemically it is similar to urobilin, but its absorption spectrum differs, and with  $\text{ZnCl}_2$  it gives no fluorescence. It is not precipitated by PbAc. In testing the spectrum the solution must be made acid with acetic acid, and the lines for urobilin must not be mistaken. The spectrum is the only means of recognition.

**The reducible body of Stokvis** is a by-product of the complete oxidation of bile pigment. It is a substance soluble in water, alcohol, alkali, and dilute acid, but not in ether or chloroform. It is not precipitated by PbAc, but is by PbAc and  $\text{NH}_4\text{OH}$ . It is characterized by the fact that if its alkaline solution be boiled with a reducing substance (e.g.,  $(\text{NH}_4)_2\text{S}$ ), the solution becomes a beautiful rose-red with an absorption spectrum. This shaken with air, the rose red disappears, and it is restored to its original color with the disappearance of the spectrum.

The *color* of the urine does not depend alone on the amount of bilirubin present, for much may be present in a pale urine and little in a very dark. In general it varies from a dark yellow to brown or even greenish-black. The most characteristic feature is the yellow foam, since in a very dark non-jaundiced urine the foam produced by shaking is pure white unless much urobilin be present. The urine has a yellow sediment and stains the filter paper yellow. As a rule, in such urine there is also an excess of urobilin and indoxyl, hence when the bilirubin disappears the color of the urine still remains dark. It also contains the nucleo-albumin of the bile. Such urines are not adapted to Heller's albumin test since the oxidized pigment will confuse one.

**Tests.**—If there be much bile in the urine it may be shaken out with chloroform, the chloroform extract poured off, evaporated, the residue taken up again with chloroform, and evaporated in a watch-glass. Rhombic prisms of bilirubin are seen which are soluble in alkali, give the Gmelin test, and on exposure to the air become green.

**GMELIN'S TEST.**—The urine is superimposed in a test-tube on crude  $\text{HNO}_3$  (sp. gr. at least 1.4). The urine is best added with a pipette and these fluids stratified as for the albumin test. The  $\text{HNO}_3$  should be faintly yellow with  $\text{HNO}_2$ , not too much nor too little so. The yellow may be increased by adding a few pine shavings to the nitric acid or diminished by adding a little urea.

If bilirubin be present, strata of colors will be seen in the urine which from above downward are green, blue, violet, red, and, just above the  $\text{HNO}_3$ , yellow.

If too much  $\text{HNO}_2$  be present, soon the whole is yellow. This test cannot be applied to a very dark urine or to a urine rich in indican. If the latter be present, the blue of indigo may with the yellow of the urine give a deceptive green. The ring has a black tone, and a fine precipitate can be seen. If in doubt the urine may be extracted with chloroform and both pigments tested for. A violet-red ring may be due to skatoxyl. Often only a green color is seen. This is necessary for

diagnosis, and most persons agree is sufficient. The violet-red, according to others, must also be present, else the test may be confused with that of lutein, which gives a blue or a bluish-green ring; but in the case of urine this pigment will not disturb. The violet-red is due to skatol and indoxyl. Biliverdin also gives this test, but the reaction occurs in a shorter time since the first oxidation step is already present. The test may fail if too much  $\text{HNO}_2$  be in the nitric acid, since the green will not be seen.

Alcoholic solutions cannot be tested, since alcohol alone will give this test. If urines are shaken out with ether, the ether must be alcohol-free, and this is not always the case.

If urobilin be abundant, the bile test will be poor, but, as a rule, it does not disturb much. The urine should be diluted to a specific gravity of 1005, and the green bile test obtained. Some prefer always to dilute, since then only the green is seen.

Lutein of the serum does not disturb, but methæmoglobin may. An abundant albumin precipitate will obscure the test, and if the albumin be removed it will carry the bile down with it. This albumin precipitate should be dried and extracted with chloroform. A trace of albumin will not disturb the test, and if much bile be present will even improve it, since so much will be carried down with the precipitate, but if only a trace of bile be present and a trace of albumin, the latter must be precipitated, dried, and extracted.

The Gmelin test is said to indicate as little as 1 : 80,000.

After the ingestion of antipyrin it is said that the test is positive.

ROSENBACH'S TEST is the best modification of Gmelin's test, being the most sensitive. Much urine is filtered several times through a filter paper, which will retain the bile-stained elements of the sediment. The filter is then partially dried with dry filter paper and one drop of yellow  $\text{HNO}_3$  dropped upon it. Rings will be seen which will present the above-mentioned play of colors, the external one being green. The urine should be somewhat acidified with  $\text{HCl}$  before filtering. If the paper be allowed to dry, it should be again moistened with water before the test. Instead of filter paper Dragendorff uses a porous porcelain plate.

A test considered by some very delicate is the following. A test-tube is filled full of urine, and 2 cc. of chloroform and 3 drops of  $\text{HCl}$  added. It is then thoroughly mixed. The bile pigment, which acts as an acid, is set free from its alkali combination by the  $\text{HCl}$ , and, being more soluble in this condition in chloroform than water, is extracted by the chloroform, the free pigment being insoluble in water. The chloroform is then poured off into another test-tube and equal amounts of water added. One drop of  $\text{NaOH}$  is then added, transforming the free pigment to an alkali salt, which is again soluble in water. The  $\text{H}_2\text{O}$  solution is then tested with nitric acid, or the chloroform extract may be evaporated in a watch-glass and the crystals studied.

By another method the urine is rendered alkaline with  $\text{NaOH}$ , or soda, and precipitated as long as a colored precipitate falls with  $\text{BaCl}_2$  or  $\text{CaCl}_2$ , or the hydroxides of these metals. The yellow precipitate is then filtered off and boiled with alcohol plus a few drops of dilute  $\text{H}_2\text{SO}_4$ . A beautiful clear green solution is obtained. If no pigment is present it is colorless; if chrysophanic acid is present, orange-yellow. This test is positive when other tests are negative.

The bilirubin may also be extracted with chloroform from acid urine (add a few drops of  $\text{HCl}$ ). An emulsion should be avoided, however, by not shaking too vigorously. In case it be necessary to test the urate sediment, and this may contain



all of the bile in the urine, the sediment is dissolved with soda and the solution tested for bilirubin.

**HAMMARSTEN'S TEST.**—An acid mixture is used, consisting of 1 part 25 per cent.  $\text{HNO}_3$  and 19 parts of 25 per cent.  $\text{HCl}$ . This reagent is allowed to stand until yellow. To 1 part of this are added 4 of alcohol; this mixture is made fresh before each test. To a few cubic centimetres of this fluid are then added a few drops of a bilirubin solution. At once is obtained a permanent beautiful green color. If more acid be added we get at will the other colors, even the yellow choletelin. But for urine this test must be modified somewhat. Ten cc. of urine are placed in a 15 cc. tube of a centrifuge, a few cubic centimetres of  $\text{BaCl}_2$  solution added and the mixture centrifugalized for from one-half to one minute. The supernatant fluid is then poured off. 1.2 cc. of the above acid reagent are then added to the sediment, this shaken well and centrifugalized for half a minute. (If  $\text{CaCl}_2$  be used, this last centrifugalization is not necessary.) A green solution is obtained. The test is very delicate, being positive if there is 1 part of the pigment in 500,000 to 1,000,000 parts of urine.

**HUPPERT'S TEST.**—Ten cc. of urine are made alkaline with soda and  $\text{CaCl}_2$  added as long as a precipitate is formed. This is filtered in a small filter and washed with water. The filter with the precipitate is then placed in a porcelain dish and acid alcohol (5 cc.  $\text{HCl}$  in 100 cc. of alcohol) added. This is heated and gives a green to a blue colored solution. This test is advised should indican be abundant or the urine dark in color.

**NAKAYAMA'S MODIFICATION OF HUPPERT'S TEST.**—The reagent used consists of 95 per cent. alcohol, 99 parts; fuming  $\text{HCl}$ , 1 part; and 4 gms.  $\text{Fe}_2\text{Cl}_6$  per litre of the above mixture. To 5 cc. of acid urine are added an equal amount of 10 per cent.  $\text{BaCl}_2$  solution and centrifugalized. The supernatant clear fluid is poured off, to the precipitate are added 2 cc. of the above reagent, and the fluid is then heated to a boil. A green solution is obtained, or a bluish green, which on the addition of yellow  $\text{HNO}_3$  becomes violet or red. The test is said to be positive for 1 part of bilirubin in 1,200,000 parts of urine; that is, it is almost twice as delicate as Huppert's test.

The following very important test or modifications of it has gone under four different names, **TROUSSEAU'S**, perhaps, having priority. The urine, acidified if necessary with acetic acid, is mixed with a tincture of iodine, or a contact test made, the iodine tincture being superimposed upon the urine. A fine emerald-green color is obtained (which is not biliverdin but a substitution product of bilirubin with iodine). This test is more sensitive than Gmelin's; it is even more delicate if the tincture of iodine be diluted 1 : 10 with alcohol (hence a 1 per cent. iodine solution) and the urine be overlaid with this. A green ring appearing at once or in one minute will indicate bile (Rosin). In this test there is no confusion with indoxyl. It is said, however, that some normal urines will give a positive test. (Cl or Br may also be used.)

**STOKVIS'S CHOLECYANIN TEST.**—This test is a good control if bile be present with much other pigment, but it is not as delicate as some of the above. To 20 to



30 cc. of urine are added 5 to 10 cc. of ZnAc solution. A little soda is added to reduce the acidity. Or, 20 per cent. ZnCl<sub>2</sub> solution may be used. It is then filtered. The precipitate contains all of the bile. This is dissolved in NH<sub>4</sub>OH. The bile pigment is now in the form of cholecyanin. The solution is neutralized, is of a blue-green color, with a red fluorescence and a characteristic three-band spectrum.

Many of these tests are good. Some when bile alone is present, others in the presence of other pigments also.

Certain substances may be in the urine which it is important should not be mistaken for bile, as after the use of rhubarb, senna, and san-tonin. These urines become red on the addition of an alkali, but the color is restored if the urine be again acidified.

Microscopically, it is important to recognize the crystals of bilirubin. They are commonly present in jaundiced urine if concentrated for leucin and tyrosin. The urine is rendered acid with HCl and allowed to stand in the cold. Bilirubin will precipitate out in intensely brown sheaths or rhombs often with rounded edges; their color should prevent any confusion.

It is sometimes desirable TO REMOVE BILE from the urine. This may be done by extracting a urine acidified with HCl with chloroform, or by briefly boiling with a little animal charcoal. This latter method should be carefully used, since other substances, perhaps the one sought for, may also be removed.

KMnO<sub>4</sub> in acid solution destroys the bile pigments perfectly. Two drops of HNO<sub>3</sub> or HCl per 1 cc. of urine are added, and 2 drops of 4 per cent. KMnO<sub>4</sub>. The urine is then warmed and shaken a little.

Bouma <sup>48</sup> recommends the following QUANTITATIVE DETERMINATION FOR BILE: To 10 cc. of fresh urine are added 2 cc. of 20 per cent. CaCl<sub>2</sub> solution. The urine is then almost neutralized with NH<sub>4</sub>OH. The slightly acid urine is then centrifugalized, the fluid is poured off, the sediment shaken up with water and centrifugalized again to wash the sediment. The fluid is entirely decanted, and 5 cc. of a mixture of 4 cc. of absolute alcohol and 1 cc. of Obermayer's reagent (1.5 gms. of Fe<sub>2</sub>Cl<sub>6</sub> in 1 litre of HCl, sp. gr. 1.15) are added. This is then poured into a test-tube, and compared with a set of six standard tubes to match the biliverdin which has been formed. If much bilirubin (more than 100 mg.) be present, the urine is diluted with normal urine (thus not diluting the phosphates).

**Melanin-Melanogen.**—In the case of melanotic tumors this substance or substances (Mörner) may be present in the urine. The chromogen is colorless, but the urine on standing, or after the addition of an alkali or oxidizing agent, turns black, beginning at the top; it may be intensified by adding HNO<sub>3</sub> or Fe<sub>2</sub>Cl<sub>6</sub>. It is insoluble in chloroform, which prevents its confusion with indoxyl. It may be

<sup>48</sup> Deutsche med. Wochenschr., 1904, No. 24.

present as an amorphous sediment. It is decolorized by boiling with  $\text{NHO}_3$ .

**Rosenbach's Reaction.**—The urine is boiled, adding from time to time, drop by drop, strong nitric acid. The urine takes a Burgundy-red color and the foam a bluish-red. The foam must be of this color, since the red of the urine may be due to urobilin. (An excess of  $\text{HNO}_3$  gives a yellowish-red to yellow color with a yellow foam.) If, then, soda or ammonia be added drop by drop we get a bluish-red precipitate soluble in excess and a brownish-red solution. This test is said to be due to indigo-red (Rosin), perhaps also skatoxyl-red. It has the same significance as the indoxyl reaction.

**Bile Acids.**—Glycocholic and taurocholic acids are denied to be constituents of normal urine, as was formerly believed, but they may occur in large amounts in jaundice, especially the obstructive, although none may be present even here, and in toxic jaundice they appear but in traces. Formerly their presence was supposed to speak against the latter, but they are of little value in this differential diagnosis. Their direct detection in the urine is impossible unless 0.5 per cent. of the acid be present.

**SEPARATION.**—*Thierfelder* recommends that the urine be concentrated to a small volume, the residue extracted with strong alcohol, and filtered. The alcohol is evaporated and the urine precipitated with basic  $\text{PbAc}$  and  $\text{NH}_4\text{OH}$ . The precipitate is washed with water, dried, treated with boiling alcohol several times, and filtered hot. The filtrate, plus a few drops of soda solution to decompose the  $\text{Pb}$  salts, is evaporated to dryness, the residue extracted with absolute alcohol, filtered, and a great excess of ether added and allowed to stand. An amorphous precipitate of the sodium salts of these acids is obtained, later crystalline. The precipitate is dissolved in water and tested by *Pettinkofer's* test.

**Tyson Method.**—From 180 to 240 cc. of urine are evaporated to dryness on the water-bath. An excess of absolute alcohol is added to the residue and filtered. To the filtrate are added from 12 to 14 volumes of ether, which precipitates the bile acids. They are then filtered off, dissolved in distilled water, and decolorized with animal charcoal.

**Pettinkofer's Reaction.**—To the solution in a test-tube is added a little cane-sugar and then slowly drop by drop concentrated  $\text{H}_2\text{SO}_4$ , shaking well all the time and warming to  $70^\circ \text{C}$ ., not over, and cooling if necessary. We get first a precipitate of cholic acid, which redissolves. Then when more  $\text{H}_2\text{SO}_4$  is added there is obtained, first a cherry-red, then a beautiful purple color, which in eight days is a bluish-red. This color is due to the reaction of cholic acid and the furfurol, which is formed by the action of sulphuric acid on cane-

sugar. The purple-red solution may be diluted with alcohol, and shows a characteristic absorption spectrum. Confusing substances which may be present are albuminous bodies, many bodies easily decomposed by  $\text{H}_2\text{SO}_4$ , many pigments, amyl alcohol, and oleic acid; but in all these the absorption spectrum fails.

*Udránzky's Test.*—This is the best test. Furfurol is used directly. To 1 cc. of the solution to be tested is added 1 drop of a 0.1 per cent. watery furfurol solution. This is underlaid with 1 cc. of concentrated  $\text{H}_2\text{SO}_4$  and cooled to restrain the reaction. In the presence of only 0.033 mg. of cholic acid is obtained a red color which after standing becomes a blood-red. If 0.05 mg. be present, one gets a distinct absorption line in the spectrum. The spectrum must always be examined for confirmation. A 10 per cent. cane-sugar solution will give as good a test as a 0.1 per cent. furfurol solution. The red color must have a clearly bluish tinge.

If the cane-sugar be used in excess it is burned to a brown or a black. An excess of furfurol gives an orange color. Oxidizing bodies prevent the reaction.

Strassburger's test may be sometimes applied directly to the urine. It is useless, however, since normal urine will give a confusing color, and jaundiced urine is not suitable because of its color. This test is easy if a little bile be added to normal urine, but it is almost impossible to apply in a jaundiced urine. To the urine was added a little cane-sugar and then filtered; the filter paper was dried, and one drop of pure sulphuric acid added. In about a quarter of a minute is seen a violet spot. This will detect 0.3 gm. in 1 litre.

Skatoxyl and indoxyl will give a violet color, and with concentrated normal urine beautiful positive tests may be obtained.

If one wishes to be sure of bile acids they must be isolated as lead salts, the other tests being unsatisfactory.

*Hay's test* for bile acids is said to be very easy, sensitive, and accurate, being given by no other body occurring in the urine, and more delicate than the Pettinkofer test. On the surface of the urine (which has been cooled, if necessary, to a temperature not higher than  $17^\circ \text{C.}$ ) is sprinkled a little finely powdered sulphur. If the sulphur sinks at once, it indicates 1:10,000. If it sinks after shaking gently and waiting one minute, 1:40,000. It is given by even 1:120,000. The bile salts are said to lower the surface tension.<sup>49</sup>

**Diazo Test.**—Certain diazo bodies combined with aromatic compounds give a colored reaction. A test depending on this is recommended by Ehrlich for clinical use. What body or bodies give it in the urine are unknown, but the empirical value of the test is granted. Since there are a great many diazo tests for various bodies, one must be careful in modifying this one of Ehrlich.

Fluids:

(1) One-half per cent.  $\text{NaNO}_2$ . This should be quite fresh.

<sup>49</sup> Beddard and Pembrey, Brit. Med. Jour., March 22, 1902.

(2) Five parts of sulphanilic acid, 50 of HCl, 1000 of distilled water.

To 250 cc. of the second are added 5 cc. of the first solution. Only a fresh mixture (not over one day old) should be used. Equal parts of the urine and this mixed reagent are shaken together until considerable foam is produced and ammonia is then quickly added in excess; usually it is added drop by drop, although we are warned not to thus modify in the least the original technic. If the test be positive, the urine will take an intense red, the foam a more or less brilliant rose-red color. A brown color is often obtained in normal urines, and unless the color is a definite rose the test should be considered negative; a salmon tint is not positive. If a positive test be allowed to stand, a precipitate should form, on the upper surface of which is a zone of dark greenish-black, or violet. In case the color of the foam is doubtful,—if, for instance, after shaking the red disappears,—we are recommended to wait twenty-four hours for this precipitate. Others consider that the sediment is a less delicate indicator than is the color of the foam, and is not essential to a positive test, hence neglect it.

Some say the red color must remain in the fluid until the foam is gone.

In Green's modification 100 parts of solution 2 are used with 1 part of the nitrite. This renders the test more delicate, since fewer unexpected positive results are obtained. With strong enough reagents every urine will react positively.

Sulphanilic acid is usually used for the reagent, and yet Zunz prefers the paramido-acetophenol of Friedenwald's formula:<sup>50</sup>

Paramido-acetophenol, 50 gms.  
Conc. HCl, 50 cc.  
Water, q. s. ad 1000 cc.

Four drops of a 0.5 per cent. solution  $\text{NaNO}_2$  are added to 10 cc. of the above solution, and this to 10 cc. of urine. The mixture is then shaken, about 3 cc. of ammonia added, and the color of the foam observed. It is more delicate and intense than the sulphanilic acid. This author prefers to add the ammonia all at once, and not drop by drop. He considers the foam as the more important, and the precipitate of less value, not sufficient to make the test positive should the foam be negative. The disturbing bodies may many of them be removed by shaking the urine out with amyl alcohol, which must itself be then driven off on the water-bath.

The test is further modified by Guillemin. Fifty cc. of HCl are added to 1 litre of saturated aqueous solution of sulphanilic acid.

<sup>50</sup> New York Med. Jour., 1894, p. 745.

Two and one-half cc. of urine plus an equal amount of this reagent are mixed, and then are added two drops of  $\text{NaNO}_2$  solution. It is then well shaken and from 7 to 10 drops of  $\text{NH}_4\text{OH}$  added.

Lamanna makes the solutions with absolute alcohol instead of water.

- Solution I.    50 cgms. sulphanilic acid;  
                  5 cc.  $\text{HCl}$ ;  
                  100 cc. absolute alcohol;  
                  5 cc. glacial acetic acid.
- Solution II.    50 cgms.  $\text{NaNO}_2$ ;  
                  50 cc. absolute alcohol.

To 5 cc. of urine is then added 1 cc. of  $\text{NH}_4\text{OH}$ . The reagent mixed as in the other tests is then added drop by drop. If the test is not positive, a few more drops of the  $\text{NaNO}_2$  may be added.

Several methods of QUANTITATIVE DETERMINATION have been attempted. König places in a burette 25 cc. of filtered urine plus 5 cc. of  $\text{NH}_4\text{OH}$ . In a second burette is a mixture of 50 cc. of the sulphanilic acid solution and 1 cc. of  $\text{NaNO}_2$  solution. Into a flask are measured 5 cc. of the urine solution, and then from the other burette the mixture is added drop by drop until a red color appears in the foam and fluid which just persists after shaking.—Nizzoli determines it quantitatively by diluting the urine until the test is just positive. This is the method preferred by Zunz, who considers, however, that the determination takes more time than it is worth.

The urine soon loses its property of giving a positive test, but after a few days of ammoniacal fermentation the test reappears.

If necessary to keep the urine several days before testing it, ether may be added.

Some prefer to concentrate the urine on a water-bath to a syrup (Michaelis) and get a positive test in some cases in which the urine gave none. Zunz has done the most of his careful work with such concentrated urines. That this does not always help matters has been shown by Imhoff, who found in the experimental tuberculosis of rabbits that the concentrated urine may give a brown foam, but if diluted to its previous volume the foam becomes a brilliant red. In the case of the human urine similar observations have been made by Dr. Hirschfelder, who tests the undiluted and the diluted urine as a routine. In work done in this clinic we have been in the habit of testing the diluted, the concentrated, and the unaltered urine. I am told that certain urines giving no test according to usual technic give a good one if only one-half volume of reagent is used.

What the body is which gives the red color when combined with a diazo is not known. One of the interesting recent suggestions is that of Bondziński, who found alloxypoteinic acid in all normal urines

which, since it will give the test, he suggests as the cause. Clemens replied that this was not the important body since the body giving the diazo test is sulphur-free.

**OCCURRENCE.**—In health the test is never positive. Ehrlich has divided diseases into four groups. The first is that of non-febrile diseases, such as advanced heart disease, chronic hepatitis, carcinoma especially of the pylorus, leukæmia, marasmus senilis, malarial cachexia, tuberculous abscess, etc. In these it is rarely positive.

**Febrile Diseases.**—These Ehrlich divides as follows:

(1) Those in which the test is almost never given,—*e.g.*, acute articular rheumatism and meningitis.

(2) Diseases in which it may or may not be positive,—as pneumonia, scarlet fever, diphtheria, erysipelas, and phthisis.

(3) Those in which it is almost constantly present,—typhoid fever and measles.

Lobligeois in scarlet fever found the test positive in 42 of 52 cases, and in but 3 of 137 cases of diphtheria. He considers it therefore important in the diagnosis of cases of diphtheria with a scarlatinal rash—*e.g.*, the serum erythema. Brunschwig found that in children the reaction is always positive in typhoid, often in scarlet fever, quite often in measles, rarely in pneumonia, and never in whooping-cough. Tropea and Brancati consider that the test is not very valuable, since it occurs so variably in some diseases, so often in others, and, they claim, in some normal persons. In disease they suppose it to depend upon the virulence of the organism and the products of the breaking down of body tissues.

Ehrlich considers that in the first two groups of fevers, those in which it is almost never, and in those in which it is sometimes, present, the positive reaction means a poorer prognosis. In suspected typhoid fever it is agreed that its continued absence speaks strongly against that diagnosis, also that its reappearance allows of a differentiation between a relapse or recrudescence of the typhoid and a fever due to a complication. Johnson found it present in over 80 per cent. of his cases. Montier found the test present in all cases of the pulmonary type of typhoid fever. Déléarde and Hautefeuille<sup>51</sup> found the test positive in severe cases of typhoid fever, and considered that drugs had no influence, nor did it bear any relation to intestinal putrefaction. Phenol is an important body to inhibit its appearance. Others consider that in typhoid fever it is of no value, inasmuch as it is often negative in the early stage of the disease when it is most needed.

In phthisis it is supposed to indicate a bad prognosis, although the previous opinion of Michaelis that such cases were always fatal is not borne out by the experience of others. Boissière found it in 18 of 130

<sup>51</sup> Compt.-rend. Soc. Biol., vol. liv. p. 279.

severe cases. There is some reason to think that in tuberculosis it is due not to the tuberculosis but to some secondary infection.

It cannot be used to distinguish between typhoid fever and miliary tuberculosis, since it is often positive in both conditions. It occurs also in puerperal fever and in actinomycosis of the lung.

The work of Zunz<sup>52</sup> is of particular interest to us, since it seems to have been done with exceptional care. His conclusions are that the value of the test is limited to the early diagnosis of typhoid fever and to the prognosis of tuberculous pneumonia, but that in the latter disease a positive reaction does not mean a hopeless prognosis; that it is of diagnostic value in early cases of measles, and speaks in favor of tuberculosis in cases of peritonitis, pleurisy, and nephritis; that it is often present in erysipelas; that if present, the prognosis in a case of cancer or sarcoma is more serious; that in cases of pneumonia and pyothorax (non-tuberculous) the test means merely disturbed metabolism; that in certain cardiac affections it speaks in favor of a reserved prognosis; and, in conclusion, that it is a useful test, although its value has been much exaggerated.

Many consider that the ingestion of certain drugs prevents the test, as, for instance, phenol, salol, benzonaphthol; not that these bodies inhibit the formation of substances giving the test, but that they themselves unite with the reagent, thus preventing the reaction, and if they are extracted with amyl alcohol the test is positive. Zunz does not agree.

Plezi<sup>53</sup> found it present in typhoid from the middle of the first to the end of the third week; in measles, before the eruption and during the onset. His suggestion is that apart from these conditions it occurs in streptococcus septicæmia, which explains its presence in the angina of scarlet fever, advanced lung tuberculosis and other forms of severe tuberculosis, and in conditions with a general septicæmia.

We find the test very valuable. When present it is strong evidence in favor of typhoid fever. In our typhoid cases the test very soon is negative, the result of the diuresis we encourage.

**Ehrlich's "Egg-Yellow Reaction."**—Ehrlich has called attention to a somewhat characteristic reaction in cases of pneumonia before and during the crisis. If the diazo test be tried, the urine and the foam take a yellow color before the addition of ammonia. After it is added the color changes to a lighter yellow. Ehrlich ascribes the reaction to the urobilogen formed from the urobilin of the exudate, and thinks that it predicts the crisis. Others think its value still uncertain.

**Ehrlich's Dimethylamidobenzaldehyde Reaction.**—This is another color test proposed with the hope it would turn out of value. Dimethylamidobenzaldehyde is dissolved in equal parts of conc. HCl and H<sub>2</sub>O to make a 2 per cent. solution. From 5 to 10 drops of this are added to a few cubic centimetres of urine in a test-tube. This is then agitated a few minutes or set aside, and

<sup>52</sup> Bull. de l'Acad. roy. de méd. de Belgique, ser. iv., t. xiv. p. 553.

<sup>53</sup> Wien. klin. Wochenschr., 1903, No. 31.

then the color noted. Normal urines give a greenish-yellow, but some pathological ones a distinct cherry-red color, which constitutes a positive test. This occurs in a variety of conditions, including phthisis, typhoid, and chronic enteritis. Fresh urine must be used and not heated. Nothing of value has resulted as yet.<sup>64</sup>

#### FERMENTS.

**Ferments.**—Several ferments have been demonstrated in the urine in health and in disease, in amounts depending on the general condition of the patient. The most important of these is **PEPSIN**. To demonstrate this, pure fibrin is allowed to stand for several hours in the fresh urine. This will absorb a great deal of the pepsin. It is then removed from the urine and dropped into a flask containing dilute HCl, which is placed in a thermostat. If the fibrin digests then pepsin was present. **TRYPSIN**, it is said, has been found in the urine, but this has not been confirmed. A **DIASTATIC FERMENT** has been demonstrated in some urines, and the same is true of rennin. It is claimed that there is a ferment which decomposes urea forming ammonia bodies. Such ferment, however, needs further demonstration.

**Lipase**<sup>58</sup> is either absent normally or present only in traces. It is found in jaundice, perhaps in traces in diabetes mellitus, but is found especially in those conditions, with fat necroses (in dogs after mechanical injury of the pancreas, after tying the pancreatic duct).

**METHOD (KASTLE-LOEWENHART).**—In each of three flasks are measured 5 c.c. of urine. The second flask is boiled. To the third are added 3 drops of phenolphthalein (1 per cent.) and it is titrated with tenth-normal NaOH till faintly pink. This amount of alkali is then added to flasks 1 and 2. To each of these are then added 0.25 c.c. of ethylbutyrate and 0.1 c.c. toluene, and they placed in a thermostat at 39° C. for twenty hours. An amount of tenth-normal HCl, which is 0.5 c.c. more than the amount of tenth-normal NaOH previously added, is then added to each, they are shaken out with 50 c.c. of ether and 25 c.c. of alcohol, 3 drops of the phenolphthalein solution are added to the ether extract and the amount of butyric acid split off titrated with tenth-normal KOH.

In case 5 c.c. of urine for each flask are not available the figure obtained from the smaller amount is calculated for 5 c.c., using the formula that the amount of ferment action varies as the square root of the amount of ferment present.

<sup>64</sup> Simon, *Am. Jour. Med. Sci.*, 1903.

<sup>58</sup> See Hewlett, *Jour. Med. Research*, 1904, vol. vi, p. 377; also Garnier, *Compt.-rend.*, 1903, vol. v, p. 1064.



## CARBOHYDRATES AND ALLIED BODIES IN THE URINE.

A small amount of carbohydrates is a normal ingredient of the urine. Three have been demonstrated,—glucose, animal gum, and isomaltose. Related bodies are also present,—the paired glycuronic acid compounds, chondroidin-sulphuric acid, nucleinic acid, mucoid of the nubecula and sometimes pentose. The total output of these carbohydrates measured as glucose amounts to from 2 to 2.23 gms. in twenty-four hours. Of glucose there is normally from 0.38 to 0.62 gm. in twenty-four hours (Naunyn, 0.4 to 1.4 gms.).

The TOTAL CARBOHYDRATES, fermentable and unfermentable, may be determined as the benzoylester. The urine is made alkaline with NaOH and the phosphates filtered off. To the filtrate in a flask are added 4 cc. benzoylchloride per 100 cc. of urine, and 40 cc. of ten per cent. NaOH, and shaken gently for ten minutes (to avoid emulsion), then vigorously for twenty to twenty-five minutes, until all odor of the benzoylchloride has disappeared. It is allowed to stand a few hours, not over night, since the precipitate gets sticky and will not filter well, then filtered, the precipitate washed, dried over  $\text{H}_2\text{SO}_4$  and weighed.

The *assimilation limit* is an interesting as well as an important conception in functional diagnosis. By this is meant the minimum amount of sugar, the ingestion of which by mouth is followed by the excretion of a slight amount in the urine. A lesser amount the body can either oxidize or warehouse. Hofmeister found that galactose and lactose passed the most readily into the urine, while dextrose, lævulose, and cane-sugar passed with much less readiness.

A spontaneous glycosuria is one which appears when the patient is on an ordinary mixed diet, that is, a diet which does not contain an unusual amount of carbohydrate. A temporary spontaneous glycosuria is one which lasts only a few days. A permanent spontaneous glycosuria continues several weeks or longer and is usually diabetic. Alimentary glycosuria *e saccharo* is a glycosuria which directly follows the ingestion of a meal rich in sugar and lasts but a few hours; alimentary glycosuria *e amylo* follows a meal rich in starch and continues but a few hours. This last indicates a worse injury to the carbohydrate metabolism than does a glycosuria of the *e saccharo* group, and it is usually diabetic.

After a meal of about 200 gms. of glucose a normal person excretes as a rule no glucose, or seldom more than 1 gm.; some persons excrete none after 300 gms. Some normal persons will excrete a little after smaller amounts; for instance, after a meal of 50 gms. Glycosuria occurs with greatest ease if the sugar is given on an empty stomach. Hunger lowers the assimilation limit considerably; pregnancy also does the same. Diseases lowering the limit are cirrhosis of the liver, cerebral disease, poor nutrition, fatty liver, phosphorus

poisoning and infectious diseases, certain neuroses, exophthalmic goitre, and any condition causing diuresis.

The assimilation limit may be conveniently tested by the following test meal (Naunyn): At breakfast, coffee and milk (about 250 c.c.), and 80 to 100 gms. of bread are eaten. In about two hours 100 gms. of dextrose are taken at one time. Thus the sugar is not taken on an empty stomach. If a measurable glycosuria results the limit is pathologically lowered. If 1 per cent. of sugar is present the suspicion of diabetes is very pressing. The sugar excretion begins in about one hour, reaching maximum in from two to four hours, and lasts at the longest but eight to ten hours. The assimilation limit for cane sugar is practically the same as that for glucose (from 150 to 200 gms.). Some believe that a lowering of the limit for cane sugar has the same significance as in the case of glucose and use cane sugar in their test meal.

The sugar is not given when the stomach is empty, since it has been shown that if the sugar reaches the lower part of the small intestine it seems to be absorbed by the lymphatics, and so does not pass through the liver, but at once into the circulation and is at once excreted.

Barringer and Roper\* by repeated assimilation tests examined the condition of a series of patients five years after a transitory spontaneous glycosuria had been discovered in them. Of these, 20 per cent. had during this interval become definitely diabetic; 15 per cent. had probably, but not certainly, become diabetic; in the case of 10 per cent. there was much doubt as to whether their condition was diabetic or not; while 55 per cent. were surely not diabetic. These writers, therefore, with good reason refuse to agree with Von Noorden that all persons who show on occasions a spontaneous glycosuria are necessarily cases of latent diabetes.

To which of these two categories of transitory spontaneous glycosuria, the diabetic and the non-diabetic, a given patient belongs may be determined by testing his assimilation limit a few months after the glycosuria has disappeared, and then again a few months later.

The *hunger diabetes* of Hofmeister is very interesting. He found that if dogs under close confinement be kept on a poor diet, not starved, a certain number of them soon become diabetic and excrete 30 per cent. of the starch of their food as sugar. Naunyn made the prophecy that this would soon be found to explain the glycosuria of certain chronic diseases of man, the disease bringing about a condition of malnutrition. Later Hoppe-Seyler reported<sup>59</sup> ten cases of temporary

\* Am. J. Med. Sc., June 1907.

<sup>59</sup> Münch. med. Wochenschr., April, 1900.

glycosuria in tramps who had been under very unsuitable hygienic and dietary conditions. The glycosuria disappeared in twenty-four hours after their physical condition had improved somewhat.

**Glycosuria.**—That normally a small trace of glucose is present in the urine can be proved by isolating the glucosozone from large amounts of urine. Quantitative reduction tests before and after fermentation of the urine also indicate the presence of this body.

Theoretically, pathological amounts of glucose appear in the urine: (1) When there is a hyperglycæmia of 0.3 per cent. or over. A hyperglycæmia may be due to the ingestion of more sugar than can be warehoused or to the accumulation in the blood of glucose which the body cannot use, and which therefore the kidneys excrete. (2) When the ability of the kidneys to retain glucose is diminished, *e.g.*, after phlorizin injection. (3) When the glucose exists in some chemical combination which renders it unfit for use.

Clinically, the cases may be grouped according to Hammersten as follows:

(1) Those with a lowered assimilation limit. In this group are many with mild diabetes. Such have no glycosuria on a carbohydrate-free diet.

(2) Those with an excessive amount of glucose formed in the body. This occurs after certain experimental lesions of the brain and perhaps after certain cerebrospinal diseases. Perhaps this group includes group (6). The source of the sugar in these cases is probably albumin.

(3) Cases in which the body cannot use glucose, which therefore collects in the blood. Severe cases of diabetes mellitus belong in this group. Usually diabetics are unable to use the dextrose molecule alone, perhaps to produce the preliminary splitting of this molecule. Their ability to burn other bodies is usually normal. According to Opie these cases belong to the following group.

(4) After disease or removal of the pancreas. In dogs the glycosuria may reach 10 or 22 per cent.; the animal lives not over four to five weeks. In such experimental cases practically all the sugar ingested is excreted, and in a quite constant ratio to the nitrogen of the urine (2.8:1); in analogous cases in man not quite all is excreted. In some patients is found atrophy of the pancreas as a whole or degeneration limited to the islands of Langerhans.

(5) Glycosuria follows oxygen starvation due to any cause; suffocation, the death agony; certain poisons, as CO, curare, and amyl nitrite; narcotics, as ether, chloroform.<sup>60</sup>

(6) Certain poisons, including morphia, strychnine, and cocaine;<sup>61</sup>

<sup>60</sup> See also Brown, Johns Hopkins Hosp. Bull., May, 1900.

<sup>61</sup> See also Neubauer and Vogel, p. 92.

fusel oil,  $\text{HgCl}_2$ , acids. In this connection the work of Herter<sup>62</sup> is interesting, showing that the local application of reducing substances to the pancreas (adrenal extract and various poisons,  $\text{H}_2\text{S}$ ,  $\text{KCN}$ ,  $\text{H}_2\text{SO}_4$ ) causes glycosuria.

(7) After severe cooling of the body.

(8) Renal diabetes. After the use of caffeine or theobromine, or any diuretic which increases the secretion of the kidney (renal diabetes). This and phloridzin diabetes are the only cases of glycosuria without hyperglycæmia. Some cases of chronic nephritis have diabetes, but, as a rule, the diabetes is the primary trouble. There is a tendency for the glycosuria to lessen as the nephritis progresses. This explains the belief, on the part of some, that Bright's disease cures diabetes. This diminution of the glycosuria, in these cases, is due to an increased ability of the body to burn sugar, not to a decreasing ability on the part of the kidneys to excrete sugar.

Glycosuria follows the transfusion of normal salt solution; the injection of sugar into the blood; insults and injuries to the liver (well seen in animal experiments); rarely cirrhosis of the liver; diseases and injuries of the central nervous system, the best illustration of which is in animals, the piqure of Claude Bernard, causing hyperglycæmia of even 0.7 per cent. which lasts from six to forty-eight hours, and a glycosuria which may reach in rabbits even 6 per cent. In man a similar glycosuria follows apoplexy. It is transitory, as a rule, begins in two hours and lasts even six days, reaching 1 to 2 per cent. Glycosuria is also caused by: brain tumors, especially those of the base; dementia paralytica commonly; epidemic cerebrospinal meningitis; tabes; multiple sclerosis; diseases of the sympathetic nervous system; severe trauma of the skull, in which case it is usually permanent, beginning at once or in a year, and mild as a rule, some with an interesting relation to diabetes insipidus, beginning as this and ending as mellitus; functional neuroses; psychical causes; exophthalmic goitre; gout; arteriosclerosis and obesity. In pure diabetes, however, no such gross lesion is found.

#### QUALITATIVE TESTS FOR GLUCOSE.

**TROMMER'S TEST.**—To a test-tube half full of urine is added about one-third volume of 10 per cent.  $\text{NaOH}$  or  $\text{KOH}$  and then a 10 per cent. solution of  $\text{CuSO}_4$  in drops, until a few flakes of  $\text{Cu}(\text{OH})_2$  do not disappear on slightly shaking. The upper layer of the urine is then warmed, when at once a precipitate yellow or red in color appears at the top. When this appears the heating should at once be stopped. The reduction and the precipitate will spread through the fluid from

<sup>62</sup> Am. Med., 1902, p. 771.

above downward. The urine should always be examined fresh, and much albumin removed in all cases.

The reaction is as follows: If to pure water be added KOH and then the  $\text{CuSO}_4$ , the first drop of the latter will cause a precipitate of  $\text{Cu}(\text{OH})_2$  [ $\text{CuSO}_4 + 2\text{NaOH} = \text{Na}_2\text{SO}_4 + \text{Cu}(\text{OH})_2$ ]. These flakes of  $\text{Cu}(\text{OH})_2$ , on heating, will blacken, since  $\text{Cu}(\text{OH})_2 \cdot 2\text{CuO}$  is formed. If glycerin or the tartrates be added to the water, all of the  $\text{Cu}(\text{OH})_2$  is dissolved to a blue solution, which will not blacken on heating as it does if undissolved. If, instead of these, glucose be added to the water, the same blue solution of the  $\text{Cu}(\text{OH})_2$  is obtained. This, however, on warming is reduced, and a yellow or red precipitate falls. In the case of glucose the body giving the bright blue solution is  $\text{C}_6\text{H}_{12}\text{O}_5\text{Cu}(\text{OH})_2$ .

In the normal urine certain bodies are present which, like glycerin et al., will dissolve the  $\text{Cu}(\text{OH})_2$ . Such are the ammonia bodies, both those preformed and those resulting from boiling an alkaline urine, and albumin if present. These, however, are present not in sufficient quantity to give a clear blue solution, and only 3 to 5 drops of the  $\text{CuSO}_4$  can be added before some remains as a precipitate and that dissolved gives only a slight greenish color to the solution. If, however, to the normal urine or in the reagents, glycerin, the tartrates, or more ammonia be added, more or all of the  $\text{Cu}(\text{OH})_2$  will be dissolved, giving an azure blue solution varying in depth with the amount of  $\text{Cu}(\text{OH})_2$  added.

But the normal urine also contains reducing bodies which will reduce the copper on warming. Such are uric acid, the glycuronic acid compounds, pyrocatechin, and bile pigments if present, and always a trace of glucose. But the sum of all these equals about 0.5 per cent. if expressed in glucose. These bodies, when present in normal amount, will reduce some of the copper and give a yellowish solution, a dirty not a clear yellow, and a little will be carried down with the phosphate precipitate, tingeing it. If these bodies normally present be increased, a definite precipitate, hence a positive test, may result. But uric acid does not reduce at a temperature of from  $60^\circ$  to  $70^\circ$  C., and creatinin reduces much only after long boiling, although there is a little reduction at  $60^\circ$  C.; hence, as no high temperature is allowable in this test, these bodies should not confuse. They are very important, however, since they hold in solution the small amount of the suboxides which is always formed. The ability to hold in solution these reduced suboxides in the normal urine is much greater than its reducing ability, and hence glucose may be added to normal urine up to almost 0.5 per cent. before any precipitation occurs. The bodies holding the suboxides in solution are uric acid, creatinin, the ammonia salts, and albumin if present.

In glycosuria we have a great increase in glucose, the chief reducing body, and because of the polyuria a relative decrease in the amount of those bodies preventing the precipitation of the cuprous salts. In performing the test it is particularly important that the excess of copper should not be added since the black oxide will cover the precipitate of the cuprous salts. Normally 3 to 5 drops of the  $\text{CuSO}_4$  are sufficient to give a blue precipitate. In case sugar is present, however, the addition must continue until the first flakes of  $\text{Cu}(\text{OH})_2$  remain. The test is positive only when a yellow or red precipitate falls, yellow  $\text{Cu}_2(\text{OH})_2$  in a relatively weak alkaline, red  $\text{Cu}_2\text{O}$  in a strongly alkaline solution. (Neumayer<sup>63</sup> says it is the creatinin of the urine which causes the amorphous yellow rather than the crystalline red precipitate such as pure glucose solutions give.) When much alkali is used the creatinin is transformed to creatin. If much sugar is present, metallic copper may be deposited on the glass (it is often a problem to clean such test-tubes, and strong nitric acid is recommended). In case under 0.2 per cent sugar is present there will be no precipitate, and yet even then the test may be very suggestive, since the yellow solution will be of such a clear brilliant color. Again, the precipitation should occur under the boiling point or when the urine is just brought to that point to exclude the reduction by those bodies normally present.

For a successful test the proportions of the reagents should be rather accurate.

<sup>63</sup> Deutsch. Arch. f. klin. Med., 1900, vol. xlvii. p. 197.

Since one part of sugar can reduce about five parts of  $\text{Cu}(\text{OH})_2$ , as nearly this amount of copper as possible should be in the solution. Glucose alone, however, cannot dissolve as much cupric sulphate as it can reduce, and so glycerin, ammonia, or the tartrates are added to Fehling's, Purdy's, *et al.*, reagents, for they dissolve much cupric sulphate, which is then at the disposal of the glucose. The optimum relation is 1 part of glucose to 5 (3 to 7) of  $\text{Cu}(\text{OH})_2$  and 11 of  $\text{NaOH}$ . The excess of this last reagent is necessary, since the temperature of reduction depends directly upon it. If very little be present, a reduction may require hours of boiling. If but two parts of  $\text{NaOH}$  are present to one molecule of sugar, a few minutes' boiling is enough, while with an excess it is not even necessary to raise it to the boiling point to get a fair reduction.

Again, the best chance of a precipitation occurs when there is present a minimal amount of those bodies which hold the reduced copper salt in solution. For this reason it is advised by many, as a matter of routine, to always dilute the urine about 1:5, this strong dilution ruling out the influence of these other bodies in a much greater proportion than it diminishes the reducing power of the glucose.

If to a strong solution of glucose be added strong  $\text{NaOH}$  or  $\text{KOH}$ , then a little copper and the whole heated, a yellow or yellowish-brown or a dark-brown solution is obtained, the color varying with the amount of sugar and alkali, since there is not enough copper in solution and some sugar is destroyed as in the Moore test. This color plus that of the suboxides gives a color which much surprises the students. It is avoided by trying the test anew and adding a great deal more copper.

The best results are obtained if the copper be added before the alkali. More urine, however, must be added in case it is found that too much copper was used.

In the Trommer's test the fluid should decolorize as the precipitate forms and before the boiling point is reached. The precipitation should occur while the urine is still hot, and not after it has cooled down. When, however, only a trace of sugar is present, the precipitate may fall only after long boiling or after cooling, but such a reaction is not positive. The brilliant color of the yellow solution may indicate sugar, but in such a case, if the urine be much diluted, the precipitate may occur in the desired manner. In order to rule out a mistake arising from long boiling, some add to the boiling urine one-third volume of cold  $\text{NaOH}$  and then copper. Some of the sugar, however, will have been destroyed by the alkali before the copper is added, and hence the test is not nearly as delicate. Since a normal urine reduces some copper, and would more could it dissolve more, ammoniacal urines may give a good precipitate since they dissolve more than acid urines. It is also true that the great excess of  $\text{NaOH}$  will dissolve some of the  $\text{Cu}_2(\text{OH})_2$ , and in case of strong ammoniacal urine all of the cuprous salt may be held in solution. It does no good to add more copper, since the sugar has by this time all been destroyed. In a normal urine it is possible sometimes to get a positive test by adding an excess of  $\text{NaOH}$  and too much copper.

After warming there may be a clear yellow solution in a normal urine or a grayish-green shimmer due to a slight precipitate of the copper compounds of the xanthin bases and uric acid. The copper precipitated by sugar is crystalline, while that by the xanthin bases is amorphous.

In all copper tests albumin does not hinder reduction, but does the precipitation, and hence must be removed unless but a trace is present, when it may be disregarded.

The phosphate precipitate stained slightly yellow by the  $\text{Cu}_2(\text{OH})_2$  formed even in normal urine often deceives.

The urine may give a reduction when the glycuronic acid compounds are increased. Such follows the use of chloral hydrate, chloroform, morphine, camphor, phenol, resorcin, thymol, and menthol. A positive reduction is obtained sometimes after the use of salicylic acid, benzoic acid, chrysophanic acid, oxalic acid, salol, thallin, santonin, copaiba, rhubarb, sulphonal, chloroform, acetphenetidin, glycerin;

after poisoning with KOH,  $H_2SO_4$ , and arsenic. In alkaptonuria the test is positive. Saccharin hinders the reduction. In addition to the reducing substances mentioned are to be added allantoin, mucin, pyrocatechin, hydrochinon, urobilin, perhaps also indican.

We insist that the Trommer's test, although it is used but very little in practice in this country, shall be the one upon which the students shall practise the copper tests. The reason for this is that all steps in the process are evident, and the chances of error are very apparent, hence the difficulties of copper testing can be well learned through it. It is not so delicate as the Fehling's, and yet we have been interested to see those who have had the greatest experience in sugar work use this qualitative test as a routine matter. The reason for this is that it tells more than does the Fehling's, indicating the presence or the absence of certain bodies and in a rough way the amount of sugar that is present. If, for instance, the undiluted urine gives a barely positive test, 0.2 per cent. of sugar may be assumed, and from the amount of copper necessary to add for a good precipitation a rough approximation may be made.

*Fehling's Test Solution.*—In Fehling's solution Rochelle salt is used that there may be a maximum amount of copper in solution, at least 5 of  $Cu(OH)_2$  to 1 of glucose, and hence the optimum chance of precipitation without the possibility of a black precipitate. Fehling's solution is made from two fluids which must be kept separate, each quite permanent.

Solution A.  
Copper sulphate, 34.65 gm.  
Distilled water, q. s. ad 1000 cc.

Solution B.  
Rochelle salt, 173. gm.  
Sodium hydrate, 125 gm.  
Distilled water, q. s. ad 1000 cc.

The mixed solution will keep one day, but an old one may reduce on boiling. Equal amounts of these two fluids are mixed and brought to a boil. The urine is then added in small amounts until a precipitate is obtained, the amount of urine, however, never exceeding that of one of the solutions. The precipitate should appear at once. The mixture may be brought again to the boil, but prolonged boiling should be avoided; also a precipitate which forms after the urine has been allowed to stand does not necessarily indicate sugar. As usually performed, the amount of urine is added to the boiling Fehling's in one amount, yet by slowly adding one can guess pretty accurately the amount of sugar present. The test shows 0.08 per cent. of glucose. Although more delicate, it should be remembered that this test has all the faults of the Trommer's. A normal urine will always reduce a little, but not if the urine is first diluted so that its specific gravity is 1005 (Zeehuisen).

*Almén-Nylander's Test.*—The solution consists of Rochelle salt 4 gms., dissolved in 100 cc. of 10 per cent. NaOH (sp. gr. 1.015) warm, and saturated with bismuth subnitrate (about 2 gms. are necessary). When cooled it is filtered and kept in a dark bottle. The solution is permanent for years.



To the urine is added one-tenth volume of this reagent. The mixture is then boiled from two to five minutes. If sugar be present, the fluid will turn black and a black precipitate of metallic bismuth will settle. Should it become black after cooling, the test is not necessarily positive. If only a trace of glucose is present, the white sediment of phosphates may be only slightly gray, especially on its upper surface. The boiling should be continued for five minutes, not less, since only too often will the urine suddenly darken contrary to the expectations of the observer. Since it is difficult to boil this urine so long (by the watch), it is much better to leave the tube in a boiling water-bath. If only a trace of sugar be present, the amount of reagent used, one-tenth volume of the urine, must be accurately measured. If this test is negative we may be sure no sugar is present. If faintly positive, the test must be confirmed, since bismuth is also reduced by certain paired glycuronic acid compounds sometimes present. This test is very delicate; in fact, is given, some say, by normal urine (14 per cent. of cases). Uroerythrin may deceive, since it simulates the test; also hæmatoporphyrin. Concentrated urines may give a positive test. The test will indicate 0.05 per cent. (others say 0.025 per cent.). Any increase or diminution in the alkalinity of the fluid injures the delicacy of the test, hence it should be applied carefully in an ammoniacal urine. If the sugar is over 0.2 per cent. the yellow color of the Moore test is first seen. Rhubarb and senna will give a reduction, but before heating it will be noted that the fluid takes a brownish-red color. The test is positive after salol, benzol, sulphonal, trional, antipyrin, kairin, much quinine, eucalyptus tincture and oil of turpentine. It is also positive after a person has eaten asparagus, a fruitful source of error. All of the albumin should be removed unless it be but a mere trace, since the  $\text{Bi}_2\text{S}_3$ , if precipitated in considerable amount, is of a brownish color; if very little, a red. Ammoniacal urines are disturbing, since the  $\text{NaOH}$  replaces the ammonia, which is volatilized, leaving the solution not alkaline enough. This test is very valuable, since it is a very good control of the copper tests. Nylander's fluid is not reduced by uric acid, creatinin, pyrocatechin, hydrochinon, nor the alkapton bodies, and these are the greatest sources of error.

*Fermentation.*—This is necessary to prove that the reducing body is a sugar of three or a multiple of three carbon atoms (yet not all of these ferment). Fresh active yeast should be used. A piece about the size of a pea is added to the urine, which is then gently shaken (if shaken too hard the amount of air in solution will be increased and afterwards give a bubble suspiciously large), and the urine then filled into a fermentation tube. This is let stand at the optimum temperature of from  $15^\circ$  to  $34^\circ$  C., and the presence of gas determined in a few hours. Two control tests should always be made: the one with normal



urine to which a little glucose is added, to prove the activity of the yeast; another of normal urine alone, to prove by the absence of gas that there is no self-fermentation of the yeast. Above  $45^{\circ}$  C. there is no fermentation. The rapidity depends to a certain extent on the amount of the yeast. The amount of gas formed from a given amount of sugar, however, depends on the age of yeast, there being less formed the older it is. The maximum production of  $\text{CO}_2$  (46.5 per cent. of the sugar) is obtained only when to one part of sugar is added not more than one-half of fresh yeast. If more yeast be used self-fermentation may result. This test indicates from 0.1 to 0.05 per cent. when boiled urine is used.

Some consider it necessary to prove that the gas which was liberated is  $\text{CO}_2$ . This is easily shown by dissolving it in  $\text{NaOH}$ . Some consider it necessary to prove also that *alcohol* was formed. This is easily done by distilling the fluid, adding to the distillate a little  $\text{NaOH}$  and some Lugol's solution, then warming it, and allowing it to stand for some hours. Crystals of iodoform will form if alcohol or acetone was present in the distillate. Or to the distillate may be added a little very dilute solution of potassium bichromate and a little sulphuric acid. The fluid then on heating will turn green and give off the odor of aldehyde.

To exclude bacterial action the fermentation should occur within a few hours. Or bacterial growth may be inhibited by the addition of  $\text{NaF}$ , enough to make a 1 per cent. solution, or by tartaric acid. Many recommend boiling the urine first for about ten minutes to sterilize it and also to free it from air.

If only a trace of glucose is present there may be no  $\text{CO}_2$  seen, since the urine can dissolve some, but the positive Nylander will disappear after long fermentation.

*Phenylhydrazin*.—This test is the court of last appeal in the recognition of those carbohydrates which form with phenylhydrazin osazones of definite crystalline shape and with a definite melting point. In any case albumin must be removed, for it hinders crystallization. Ten cubic centimetres of urine are precipitated with a few drops of concentrated  $\text{PbAc}$  and filtered. One drop of acetic acid is added (or enough to acidify), then a piece of  $\text{HCl}$ -phenylhydrazin the size of a pea, and of  $\text{NaAc}$  the size of a bean. The tube is then boiled in a water-bath from one to two hours, its contents filtered hot, and the tube returned to the water-bath, which is allowed to cool down slowly. If much glucose was present there will be a deposit of yellow crystals in the form of needles arranged in sheaths. That the test may succeed, the sugar should be to the phenylhydrazin and the  $\text{NaAc}$  as 1 : 2 : 3.

V. Jaksch recommends the following: To a test-tube containing 6 to 8 cc. of urine are added two knife-points of  $\text{HCl}$ -phenylhydrazin and three of  $\text{NaAc}$ . If these salts do not dissolve on warming, a little more water is added. The tube is then put in boiling water and allowed to stand from one-half to one hour (this time prevents the mistake with glycuronic acid compounds). The tube is then put in a beaker of cool water and the crystals searched for microscopically. It were better to let the solution cool down more slowly. This method has been severely criticised.

Cipollina \* recommends the following method, which has proved itself very valuable. In a common test-tube are put 5 drops of pure phenylhydrazin (the base), 0.5 c.c. of glacial acetic acid, and 4 c.c. of urine. This mixture is boiled over a low, free flame about 1 minute, and is constantly shaken to prevent sputtering. Four or five drops of NaOH solution (sp. gr. 1.16) are then added, but the fluid must still remain acid after the addition of the alkali. The liquid after boiling again for a moment is cooled, and the characteristic rosettes of crystals will form at once or at least within 20 minutes. The best results are obtained with urine of low specific gravity.

The crystals of phenylglucosazon may be filtered out, dissolved in hot 60 per cent. alcohol, recrystallized by adding water and boiling the alcohol away. Their melting point is then determined. If pure, this is from  $204^{\circ}$  to  $205^{\circ}$  C.; when impure, from  $173^{\circ}$  to  $194^{\circ}$  C. The crystals are yellow needles in sheaves, which are difficultly soluble in water and in hot absolute alcohol, easily soluble in 60 per cent. hot alcohol, and crystallize out if water be added and the alcohol evaporated off. They are insoluble in ether, chloroform, etc., but soluble in glacial acetic acid. Their solution is lævorotatory.

A very simple method of determining the melting point is as follows (see Fig. 24): A small flask, A, is filled three-quarters full with concentrated sulphuric acid. Through a perforated stopper is inserted a test-tube, B, also one-half full of the same acid. Into this dips a thermometer, C, to which is attached a tube, D, containing the crystals. This tube has a lumen about 1 mm. in diameter and closed at its lower end, and into it are dropped the dried crystals. Very few are required. The tube is attached by a rubber band to the thermometer. The flask is then warmed slowly with a Bunsen burner and the point noted at which the crystals melt.

For other forms of apparatus the reader is referred to Menge's report (Bull. 70, Hygienic Lab., U.S.A., Oct., 1910).

This test theoretically is very delicate, showing 0.003 per cent. of sugar. But practically in urine examinations it is far less delicate, seldom if ever indicating glucose in urines which do not reduce Fehling's solution. Not all of the glucose is precipitated, the amount of precipitate depending on the concentration of the glucose and the relation between the reagents. From a 5 per cent. glucose solution the maximum precipitation obtained by Fischer was from 85 to 90 per cent. Much depends on the purity of the phenylhydrazin. The preference given to the HCl-phenylhydrazin is that it is crystalline and not a fluid at ordinary room temperature.

The glycuronic acid compounds will give the same test, but the melting point of these crystals is lower,— $114^{\circ}$  to  $115^{\circ}$  C. Various sugars give crystals. In pentose is the greatest danger of error with

\* Deut. med. Wchschr. 1901, No. 21, page 334.

the other tests, and hence the use of this is most important, since the crystals obtained melt at  $159^{\circ}$  to  $160^{\circ}$  C.

Of the sugars, it is given by all of those reducing copper, including also lactose and maltose. Those sugars which differ only in the first

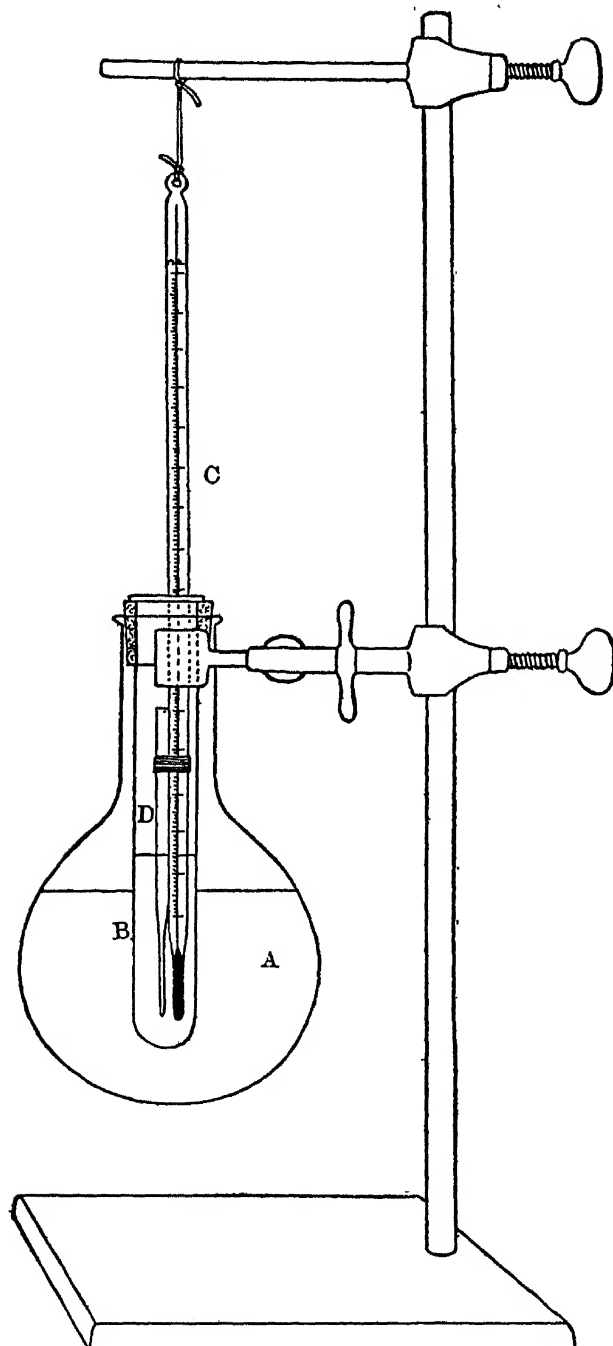


FIG. 24.—Melting point of crystals. A, flask, and B, test-tube of sulphuric acid; C, thermometer; D, fine-bore tube for crystals.

two carbon atoms, with the rest of the formula the same, as, for instance, glucose, fructose, and mannose, give this same osozone. Other

crystals are formed with acetone, hydrazin, oxalic acid, and uric acid. These, however, are not dangerous in human urine, since they do not occur in sufficient amount, and pentose is really the only one to exclude.

With the crystals also is a precipitate of brown scales and oily droplets seen even when a pure solution of glucose is used. This by-product is  $C_{12}H_{12}N_2$ , and can be washed out with chloroform or 95 per cent. alcohol and then the glucosone recrystallized from 60 per cent. alcohol. Should only brown scales or yellow amorphous precipitate or droplets be found, the test is negative, although glucose may be present.

In review it may be emphasized that there is nothing characteristic in the shape of the crystals; their best solvent is 60 per cent. alcohol; they are best recrystallized by pouring the 60 per cent. alcoholic solution into water and evaporating the alcohol; the hot glacial acetic acid solution, which is soon destroyed, may be tested with the polariscope; that in testing the melting point one cannot expect to get exactly  $204^{\circ}$  to  $205^{\circ}$  C., so much depends on the purity of the crystals and on the speed with which the temperature is raised. The latter should be done as rapidly as possible, since by slow heating the point may be much lowered. This glucosazon differs from galacosazon by the lævorotation of its glacial acetic acid solution, since that of the latter is optically inactive; otherwise they are very similar.

Zunz<sup>64</sup> considers this test one of the most important clinically, and for the further separation of the carbohydrates of the urine we give the table he recommends.

The urine reduces Fehling's.	Gives crystals with phenylhydrazin directly in urine.	Melting point of crystals about $200^{\circ}$ C.	Fermentation positive. Fermentation negative.	<div style="display: inline-block; vertical-align: middle;"> <div style="display: inline-block; vertical-align: middle;"> Dextrorotatory. Lævorotatory. </div> <div style="display: inline-block; vertical-align: middle; font-size: 2em; margin: 0 5px;">{</div> <div style="display: inline-block; vertical-align: middle;"> Glucose. Levulose. Lactose. </div> </div>
		Melting point of crystals about $150^{\circ}$ C.		
	Gives crystals with phenylhydrazin only after the urine has been warmed with dilute sulphuric acid.	<div style="display: inline-block; vertical-align: middle;"> Give orcin reaction. Do not give orcin reaction. </div> <div style="display: inline-block; vertical-align: middle; font-size: 2em; margin: 0 5px;">{</div> <div style="display: inline-block; vertical-align: middle;"> Pentoses. Isomaltose. </div>		

*The polariscope* is a very valuable instrument. In the recognition of traces of sugar care must be used, since most urines normally are slightly lævorotatory and some urines are dextrorotatory when sugar is absent (Bornträger in two morphia habitués). Albumin is lævorotatory, hence may cover a slight dextrorotation. The ordinary instrument will detect but about 0.2 per cent. of glucose. The test is of value if the urine be tested before and after fermentation.

*Rubner's Test.*—This is a modification of the Moore-Heller test. Ten cc. of urine are mixed with an equal amount of PbAc, 1 to 10 solution, and the urine filtered. Ammonia is then added drop by drop until the caseous precipitate just

<sup>64</sup> Jour. Méd. de Bruxelles, July 10, 1902.

remains. The tube is then warmed in a bath at  $80^{\circ}$  C., according to some, but heated to boiling according to Hoppe-Seyler and Hammarsten. If glucose be present a fine rose-red color results.

Or, to 10 cc. of urine add 3 gms. of the dry PbAc and dissolve by boiling; then filter. To the hot filtrate add  $\text{NH}_4\text{OH}$  and boil hard. It is well to dilute the urine that its specific gravity does not exceed 1010.

If the urine be concentrated it should be diluted one-half with water. Hoppe-Seyler recommends that it be boiled for some time before ammonia is added, and this is to be added to the boiling solution. If it be heated too strongly, however, a non-characteristic brown color appears. If it be warmed only to  $80^{\circ}$  C. the test indicates glucose, and lactose is excluded. An excess of ammonia ruins the test, hence Voit says to add 0.5 volume of PbAc solution and 0.1 volume of  $\text{NH}_4\text{OH}$ ; the urine is then filtered and the filtrate heated. To the hot filtrate is added more ammonia.

*Heat Test.*—An easy test, sometimes valuable and more delicate than one would imagine and always possible, is the following: One drop of urine is evaporated to dryness in a porcelain dish. It is then warmed gently. A yellowish-brown mass with an odor of caramels is formed at a temperature of  $190^{\circ}$  to  $200^{\circ}$  C.

*Moore's Test.*—Moore's test is one of the first used for sugar. To the urine is added one-fourth volume of KOH or NaOH. On warming is obtained, first, a yellow, then an orange, and finally a dark brown color with an odor of caramels, clearer if the urine be acidified. It may be necessary to boil for some time. It occurs slowly at room temperature. It is the least delicate test. Sometimes a normal urine will darken somewhat, also a urine rich in mucus. The nature of the colored body is not known. The names glucinic acid and melasinic acid have been suggested, one of which, probably  $\text{CH}_3\text{COCH}_2\text{OH}$ , will reduce  $\text{CuSO}_4$  in the cold.

**Choice of Method.**—Any very positive reduction test indicates sugar. If Nylander's test is only suggestive, it is of value only when we know that the urine did not contain an excess of ammonia, and that it was boiled for some time. The urine may then be polarized, but the presence of a slight amount of sugar may escape this test from the presence of laevorotatory bodies. In all cases albumin must be removed. As a routine reduction test Nylander's is the one to be recommended. Hammarsten recommends that physicians try this first. If negative, no sugar is present. If positive, try fermentation. If this is positive, glucose is the sugar. For the practitioner the fermentation alone is perhaps the best, since it leads to less confusion.

In clearing the urine for further tests it must be remembered that glucose is not precipitated by sugar of lead, but is almost completely by basic lead acetate.

**QUANTITATIVE DETERMINATION OF GLUCOSE.**—When sugar is known to be present and in good amount, a rough estimate of its amount is possible by the use of Naunyn's table:

- 2 litres of urine of specific gravity 1028 to 1030 = 2 to 3 per cent.
- 3 litres of urine of specific gravity 1028 to 1032 = 3 to 5 per cent.
- 5 litres of urine of specific gravity 1030 to 1035 = 5 to 7 per cent.
- 6 to 10 litres of urine of specific gravity 1030 to 1042 = 6 to 10 per cent.

In thus estimating the sugar from the *specific gravity*, and in the following calculation, using the coefficient 230, it is assumed that the

change in specific gravity is due alone to the sugar, which is not strictly justifiable, since urea and the chlorides also change somewhat.

Suppose the diabetic urine was 3 litres in amount and of specific gravity 1.030. Then  $\frac{2 \times 1.015 + 1.000}{3} = 1.010$ , the specific gravity

of normal urine if diluted to three litres (on the basis that a normal person voids two litres with a specific gravity of 1.015).  $1.030 - 1.010 = 0.020$ ,  $0.020 \times 230 = 4.6$  per cent.

In the same way 6 litres at 1.030.  $\frac{2 \times 1.015 + 4.000}{6} = 1.005$ .

$1.030 - 1.005 = 0.025$ ,  $0.025 \times 230 = 5.8$  per cent.

**QUANTITATIVE DETERMINATIONS OF GLUCOSE.**—The best modification of FEHLING'S QUANTITATIVE METHOD is that of Purdy as further modified by Sahli (*Deut. med. Wochenschr.*, Sept. 7, 1905).

Solution I. Pure crystallized copper sulphate, 4.158 gm.  
Distilled water, q. s. ad 500 cc.

Solution II. Rochelle salt, 20.4 gm.  
Pure potassium hydroxide, 20.4 gm.  
Ammonia (Sp. gr. 0.88), 300 cc.  
Distilled water, q. s. ad 500 cc.

For a determination 5 cc. of each fluid are used. These 10 cc. (of the mixed fluid) will indicate 0.005 gm. of glucose.

**Procedure.**—Five cubic centimetres of Solution I and 5 cc. of Solution II are carefully measured with a pipette into a small Erlenmeyer flask (of 75 to 100 cc. capacity), and 30 cc. of distilled water added. The flask is then heated till it just boils. It should rest on a tripod about 20 cm. tall which is covered with asbestos gauze, or asbestos sheet, or if these are not available, on an iron or nickel sheet covered with a layer of magnesia a few millimetres thick. The gas burner used should be one which allows accurate regulation of the flame. The diluted urine is added slowly from a burette, which is better held in the hand than fastened to an upright by a clamp.

When the fluid in the flask just boils the diluted urine is slowly added, the fluid just boiling all the time, until the last trace of blue disappears.

It is better to use distilled water than tap water to dilute the copper solution since the alkali may precipitate from the tap water a faint cloud of the alkaline earths.

One of the most delicate parts of the determination is the dilution of the urine. This must be done with extreme accuracy. The best results are obtained when just 10 cc. of the dilute urine were used to reduce the 10 cc. of the mixed copper solution. This means that the diluted urine should contain from 0.05 to 0.1 per cent. of sugar, that is that a urine containing 5 per cent. of sugar must be diluted fifty times. Such a dilution can be made best by measuring the urine from a burette, or accurate pipette, into a volumetric flask with narrow marked neck, and the fluid then very thoroughly shaken. The amount of dilution is determined by a preliminary rough determination, or by an estimation with the polariscope.

**BENEDICT'S METHOD** (New York M. J., Sept. 14, 1907).—This method promises to be one of the best for the volumetric determination of glucose. We continue in the author's words.

The solutions required are:

- Solution A. Crystallized copper sulphate, 69.3 gms.  
Distilled water, to 1000 cc.
- Solution B. Pure Rochelle salt, 346.0 gms.  
Anhydrous sodium carbonate, 200.0 gms.  
Distilled water, to 1000 cc.
- Solution C. Potassium sulphocyanate, 200.0 gms.  
Distilled water, to 1000 cc.

For use these solutions are mixed in equal proportions. Thirty cubic centimetres of the resulting solution are equivalent approximately to 0.073 gramme of dextrose.

*The Process.*—To thirty cubic centimetres of the mixed volumetric solution in a beaker is added two or three grammes of anhydrous sodium carbonate\* and the mixture boiled until this is dissolved. The urine to be titrated is now run in from a burette rather rapidly (not so quickly as to interfere markedly with continuous boiling) until a heavy chalk white precipitate is formed and the dark blue color of the solution begins to lessen perceptibly, whereupon the fluid from the burette is run in more slowly until the blue color just completely disappears. The last portion should be introduced in quantities of from two to ten drops (depending on depth of color remaining and the relative strength of the sugar solution) with vigorous boiling of about one fourth minute between each addition. The end point (disappearance of the blue color) is sharp and satisfactory.

A simple device to prevent the bumping of solutions during the process of titration consists in placing in the titration beaker a medium sized piece of pure, previously well washed absorbent cotton. By stirring this about with a glass rod as the titration proceeds, it is possible to entirely prevent the bumping which otherwise may become troublesome.

Certain substances (notably chloroform) may occasionally be encountered, which interfere slightly with the titration by causing a portion of the reduced copper to be precipitated as the suboxide even in the presence of the sulphocyanate. In case such substances are present the following solution (solution D) should be used in place of solution C:

The formula for solution D is

- Potassium ferrocyanide, 30.0 gms.;  
Potassium sulphocyanate, 125.0 gms.;  
Anhydrous sodium carbonate, 100.00 gms.;  
Distilled water, to 1000 cc.

This solution is used just as is solution C and will obviate any difficulties from interfering substances. Solution D does not alter the value of the copper solution in terms of dextrose, and may be used entirely in place of solution C if desired. Since, however, interfering substances are seldom, if ever, encountered, and solution C is a little more easily made up than is the other, solution C is recommended for ordinary use.

If VERY LITTLE SUGAR be present, the urine (1500 cc.) may be precipitated with sugar of lead, then the filtrate precipitated with basic lead acetate and a little ammonia. The precipitate is suspended in alcohol and decomposed with  $H_2S$ . The filtrate is then cleared with animal charcoal if necessary and evaporated at low temperature to a small volume. The amount of glucose in this solution is then determined with the polariscope. To exclude lactose and bile acids, both of which would, if present in the urine, be determined as well since they are dextrorotatory, the alcohol is evaporated off, the residue dissolved in water, yeast added, the glucose fermented; the fluid is then filtered, the precipitate being washed with alcohol, the original volume restored and again polarized.

\* Double the quantity of the crystallized salt may be used.

**Fehling's Quantitative Method.**—The formulas for these two solutions are given on page 175. Ten cubic centimetres of Solution A are reduced by just 50 mg. of glucose.

Ten cubic centimetres of Solution A, and ten of Solution B, are carefully measured into an Erlenmeyer flask of about 250 cc. capacity. About 40 cc. of distilled water are then added and the fluid brought to a boil. The urine, so carefully diluted that it contains between 0.5 and 1.0 per cent. of glucose, is added from a burette to the boiling copper solution, until the blue color of the copper has just disappeared. Then, supposing  $a$  to represent the amount of diluted urine added,

$$\frac{50}{a} \times 100 = b, \text{ the percentage of glucose in the diluted urine.}$$

The first determination is only approximate. The diluted urine is added one cubic centimetre at a time until the end reaction. Suppose that 8 cc. were not enough, and 9 cc. were too much, then a second determination is made by adding 8.5 cc. in one amount. Suppose that the copper is all reduced by the amount, then five flasks are filled with the Fehling's solution and all brought to a boil. To one 8 cc. of diluted urine are added, to the second 8.1 cc., to the third 8.2 cc., etc. Suppose the flask to which 8.2 cc. were added is just a trifle blue, that to which 8.3 cc. is clear, then 8.3 cc. of diluted urine must contain 50 mg. of glucose.

This method is one of the most difficult of quantitative determinations in clinical chemistry. The end reaction is uncertain owing to the abundant red precipitate, and the rapidity with which the cuprous salt is reoxidized by exposure to the air. For this reason the urine must be added in one amount, the fluid then brought just to the boiling point, and the flask at once removed from the flame. In a few moments the precipitate will have settled just enough to allow the color of the uppermost layer of fluid to be determined.

For Lehmann's method, see Sahli (1905) and Citron.<sup>65</sup>

**Polariscope.**—For this very important test and quantitative method the urine must be perfectly clear, so that through the tube of the polariscope when filled even the finest type may be very easily read. All albumin must be removed, since it is lævorotatory. The urine is best cleared, if possible, with Kieselguhr alone. An excess of this is added, the urine well stirred and filtered. The first of the filtrate is to be poured back into the funnel. If, as sometimes happens, this does not clear perfectly, crystals of sugar of lead are to be added and the urine filtered, or these two methods may be combined.

The crystals are somewhat preferable to the solution, which changes the volume of the urine. But some prefer to add 10 cc. of PbAc solution (25 gms. in 100

<sup>65</sup> Deut. med. Wochenschr., No. 44, 1904.



cc.) to 90 cc. urine, and this clears the urine perhaps better. Basic lead acetate cannot be used. Kieselguhr is recommended since lead acetate in any excess alters the physical properties of the fluid and does remove some glucose from urine, although none from a pure glucose solution, and yet Kieselguhr also may remove some sugar. Others recommend a small amount of PbAc and a teaspoonful of  $\text{Na}_2\text{SO}_4$  (added after the sugar of lead is dissolved).

The tube is then filled, care being taken that no air-bubbles be enclosed, and the angle of rotation measured by the scale on B (Fig. 25), using the vernier, C, for the fractions of a degree.

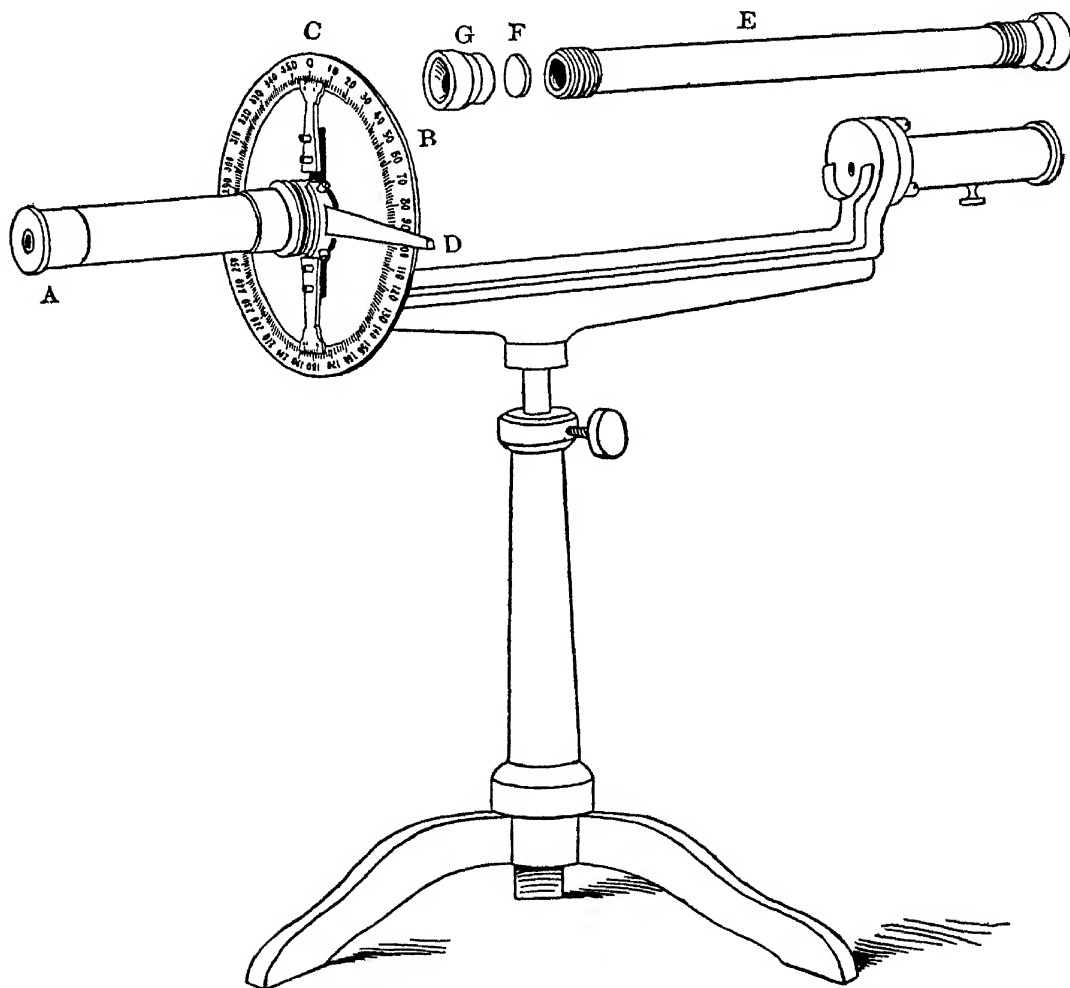


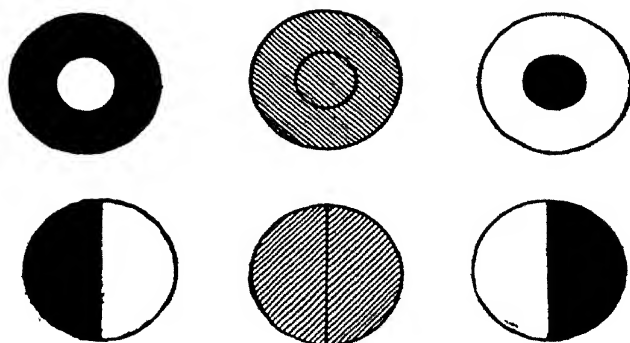
FIG. 25.—Half-shadow saccharometer. A, ocular used in focussing the field; B, graduated disk; C, vernier; D, lever for rotating analyzer; E, tube for urine; F, glass disk; and G, cap for end.

The tube, E, is first cleaned thoroughly and dried. The glass disk, F, of the ends are perfectly clean and clear. One glass is screwed in and then the tube filled with the perfectly clear urine till the meniscus is convex. The second glass disk is then slid on from the side, pushing off the excess of urine and allowing no air to enter, and the metal cap, G, screwed down over this.

The student should understand the instrument that he is using. There are many varieties on the market with slight differences in their construction and greater in their usage. It is seldom, of course, that a real polariscope is used, but

instruments modified for clinical purposes. The polariscope is an instrument which measures the angle of rotation caused by an active substance; the length of the tube is usually 10 cm. or multiples of this, and the reading is in degrees. The specific rotation of glucose is  $[\alpha]_D = 52.74^\circ$ . If a polariscope be used, therefore, the angle of rotation must be divided by 0.527 to give the percentage of sugar. Those in clinical use are usually "half-shadow instruments," and simplified by using tubes of such length—188.6 mm. (better 189.4 mm.)—that one degree of rotation will equal one per cent. of glucose. Each instrument usually has another tube, one-half as long,—94.3 mm. (better 94.7 mm.),—for highly colored urine. Another instrument which is very popular and much more convenient is the saccharometer, in which the rotation by the sugar is to be balanced by a compensating quartz wedge which is marked with an empirical scale. The great advantage of this instrument is that ordinary white light, as the Welsbach burner, can be used; in the other instrument a sodium flame only.

In using, the field must be first focussed at A and the zero point determined. This changes somewhat with the temperature, particularly in the carelessly used instrument. The tube is then inserted, the field focussed sharply, and the rotation determined. The accuracy with which this can be done will depend upon the clearness of the field which depends on the fluid and focus, the sensitiveness of the instru-



FIGS. 26 and 27.—The fields as seen in the two most common types of clinical saccharimeters. The central figures, gray fields with halves of equal illumination, are the zero points. The others are the fields with too little and too much rotation.

ment, and the brightness of the light. To find the end-point with fields of equal illumination there are two common methods. In the one case, the analyzer is rotated until a black band seems to cross the division of the fields. This shadow, purely subjective, is yet of great value. It always appears a little too soon, therefore an average of the readings made from both directions should be used. In the second method the analyzer is slowly turned, always in the same direction, the eye being used but for a few seconds at a time, until the end-point seems to be just reached, that is, where there is no perceptible difference between the two fields. This point will always be attained a little too soon, and in amount equal to the sensitiveness of the instrument. Hence, also, several readings should be made, turning from both directions, and an average taken. In all cases it should be remembered that

the eye should be used but for a few seconds at a time, not over fifteen, to prevent fatiguing the retina. The depth of illumination of the whole field should be judged, and not of contiguous portions.

The half-shadow instruments, modifications of the Laurent, have been constructed so sensitive that they allow one to appreciate a difference of only  $0.02^\circ$ , but it should be remembered that the principle on which the instrument rests is itself inaccurate to about 0.2 per cent., hence one gains very little from these very minute readings and some very careful work has only the appearance of accuracy.

The ends of the tube must be planed at a right angle to its long axis. If their axis forms an angle of over ten degrees its use is impossible. This is easily recognized by putting a tube in the instrument, focussing carefully, and then revolving the tube. The same effect is produced as if the analyzer were rotated. Leather washers are necessary to prevent too much tension of the metal cap upon the glass disk covering the end. If this glass is subjected to too high tension, it becomes doubly refractive and a similar error arises. For this reason before any readings are made the tube should be rolled between the fingers. If this causes the two fields to change in relative intensity, it may indicate one of the two above mentioned errors. If the whole of the field is not equally sharp the solution is either not homogeneous or the tube is dirty. This also is recognized by rolling the tube.

The normal urine is slightly lævorotatory ( $0.005^\circ$  to  $0.18^\circ$ ). A trace of sugar may therefore be present if the reading is practically zero. In some cases the urine is dextrorotatory when glucose is not present. Such were two cases of morphia habit (Börntrager). A polariscope is an instrument for the laboratory; for the practical man it is a great (though expensive) aid in quantitative work. Albumin must be removed, it being lævorotatory. Should the worker make up a glucose solution and test it with the polariscope, he must remember to use a solution which has stood for at least a day, since glucose shows a birotation when first dissolved.

*Fermentation: Specific Gravity Method of Roberts.*<sup>66</sup>—The amount of sugar is estimated from the difference in specific gravity before and after fermentation. The urine should be acidified, if necessary, with tartaric acid. The specific gravity is first carefully determined, using a very accurate ærometer and paying due regard to the temperature of the fluid. A piece of washed yeast the size of a hazel-nut is then added and the urine allowed to ferment at from  $15^\circ$  to  $35^\circ$  C. (a temperature of  $34^\circ$  C. is the best) until it gives no further qualitative test for sugar. This takes from twenty-four to forty-eight hours. The sediment is brought into suspension and the specific gravity again tested. The difference in the specific gravity multiplied by 234 gives the percentage of sugar. It is better to use the pycnometer method of determining specific gravity, since the ærometrical method, which at the best is poor, is hardly delicate enough for this work. With accurate work the results are correct to 0.1 per cent. Albumin need not be removed. The sugar should be at least 0.5 per

<sup>66</sup> Edinb. Med. Jour., October, 1861, p. 326.

cent. A greater accuracy is therefore claimed for this than for Fehling's method.

*Fermentation: Gas-Volumetric Method.*—The Einhorn method of determining the amount of sugar by the amount of carbon dioxide produced is thought by many to be a failure, since the amount of gas produced depends on the amount of yeast, its activity, the temperature, and many other factors; yet it is possible to control well all of these factors, and we have seen excellent results with it. Lohnstein's<sup>67</sup> instrument is said to be accurate and to give the result in six hours.

The Roberts method is the best for the practitioner who has not a polariscope nor the time for the titration. The factor used in determining the per cent. will be found to vary in various text-books, but the one to use will depend on the method employed, each modification necessitating a different coefficient. Unless one is careful in the details, an error as high as 5 per cent. of the total amount may be made.

**Levulose** is a sugar widely spread in the vegetable kingdom, particularly among the fruits. It is often present in the urine of diabetics, but associated with glucose. There are, however, a few cases of pure lævulosuria on record (Naunyn) in which this sugar was present in about 1 to 2 per cent.; but, as a rule, there is less than 1 per cent. Rosin and Laband reported recently<sup>68</sup> an interesting case of pure lævulosuria (0.6 per cent. in the urine. There was a lævulosæmia of 0.5 per cent. even when the urine was negative), and uninfluenced by the ingestion of even 100 gms. of levulose or glucose. It is interesting that it is excreted, since levulose is the sugar most easily used by some diabetics, but by no means by all. If large doses are given, it is excreted almost entirely as glucose. In non-diabetic persons lævulosuria may occur spontaneously.

Most of the reported cases of lævulosuria are doubtful, since other lævorotatory bodies were not excluded. Lævulosuria is to be suspected when the percentage of sugar determined by polarization is less than that by titration, and the lævorotatory body is fermentable. In sixteen cases Rosin and Laband found a lævorotation (titration minus polarization) of from 0.3 to 1.7 per cent. (as glucose). No acetone was present.

The most important lævorotatory sugars are laiiose and fructose. Fructose gives reactions very similar to those of glucose; it reduces copper somewhat less readily (10 per cent.), ferments, and has an angle of lævorotation of uncertain amount. Its characteristic test is that of Seliwanoff, which it is well to use in all cases of diabetes, yet

<sup>67</sup> Münch. med. Wochenschr., 1899, No. 50.

<sup>68</sup> Zeitschr. f. klin. Med., 1902, vol. xlvii. p. 182.

Rosin, also Fr. Müller,<sup>69</sup> warn against the test, stating that it must be confirmed, since glucosamin gives the same. A moderately dilute hydrochloric acid (1 volume of HCl to 2 volumes of H<sub>2</sub>O) solution of resorcin is warmed and a little levulose added; the fluid at once becomes a beautiful red color, due to a precipitate which is soluble in alcohol. Levulose, if warmed with a concentrated alcoholic solution of resorcin, gives a brick-red color. It gives the same osazone as glucose. It is a more fragile body than glucose.

The other lævorotatory bodies of the urine which must be excluded are albumin, glycuronic acid compounds,  $\beta$ -oxybutyric acid, and cystin. If the lævorotation disappears on fermenting, the strong probability is for levulose. To be perfectly sure, the sugar must be isolated.<sup>70</sup>

ALIMENTARY LÆVULOSURIA has been much used as a test in the functional diagnosis of liver disease. Strauss<sup>71</sup> found that the ingestion of 100 gms. of levulose was followed by lævulosuria in 90 per cent. (26 of 28 cases) of cases with hepatic trouble, and in but 10 per cent. (6 of 58) of normal men. Ferrannini and Bruining also considered the test valuable. Landsberg<sup>72</sup> could get the test in but 9 of 21 cases (not severe ones), and in four of seven normal persons. He therefore doubts its value.

Lactose is found in the urine of women during lactation, in which case stasis in the lacteal glands is the cause, and in that of patients who have been long on a milk diet. In feeding experiments it is present after the ingestion of 100 gms. as a rule. In diabetics Voit has found that if lactose be fed they excrete glucose, while in the case of lactating women the reverse is true. The amount present is usually small, but may be as high as 2 to 3 per cent. In the case of lactating women it has been found in the urine of 115 of 148 cases (Ney). Others report it in all cases. It reaches a maximum on the second to the fourth day after delivery.

Lactose is dextrorotatory (52.5°). Lactosazon crystallizes in small yellow prisms arranged in spheres and with a melting point of 200° C. Its reduction tests are like those of glucose, but copper is reduced somewhat less actively, and silver nitrate (ammoniacal) is reduced in the cold. Nylander's test is positive. If a solution of lactose is boiled several hours with dilute mineral acid, the lactose is inverted to galactose and glucose. It does not ferment with ordinary yeasts, though some will ferment it without the production of CO<sub>2</sub>. Its presence is to be suspected when the copper and bismuth tests are positive yet somewhat slow, and the fermentation and phenylhydrazin

<sup>69</sup> Deutsches Arch. f. klin. Med., 1904, p. 1630

<sup>70</sup> See Peligot method, Compt.-rend., vol. xc. p. 153.

<sup>71</sup> Deutsch. med. Wochenschr., 1901, Nos. 44 and 45.

<sup>72</sup> Ibid., August 6, 1903.

are negative. The urine should first be sterilized else the bacteria in the yeast and urine will split the lactose, giving a fermentable sugar. If the urine does not ferment, yet reduces copper, lactose or pentose may be suspected.

Rübner's test is valuable. The urine is boiled with an excess of sugar of lead from three to four minutes, when the solution becomes yellow or brown. To the hot fluid is then added ammonia as long as the precipitate which forms will still dissolve. An intense brick-red fluid is obtained which settles later as a copper-red precipitate with a colorless supernatant fluid. This test is positive if the lactose be from 0.05 to 0.02 per cent. The test is perhaps best performed by adding 3 gms. of PbAc to 10 cc. of urine. The precipitate is then filtered off and the filtrate tested. If the specific gravity of the urine be over 1020 it is best to dilute one-half. If the test be performed as recommended for glucose, that is, the solution warmed but not boiled, no red color is obtained—only a yellow, coffee-brown or red, according to concentration. Glucose gives a red solution with a yellow precipitate. Maltose, a little yellow, and levulose, no color at all. To prove that it is lactose present the sugar can be isolated.<sup>73</sup>

**Pentoses.**—The pentoses, sugars with a chain of five carbon atoms, occur widely in nature, not as such, however, but as products by hydrolysis of more complex carbohydrate molecules, which are very important in the vegetable kingdom. In the herbivora the pentoses play almost the same rôle as the hexoses in man, being glycogen-builders. In the animal kingdom they play an important part as the carbohydrate nucleus in the nucleo-proteid molecule of certain organs, the pancreas, thyroid, thymus, brain, spleen, and liver.

Three types of cases are separated (Janeway). The first type, or *alimentary* pentosuria, occurs in all normal persons after the ingestion of considerable amounts of fresh fruit juice, or of beer. In these cases the pentose is always optically active. These sugars, when ingested pure, pass the easiest of all into the urine, and in the case of xylose may be demonstrated even after a dose of 50 mg. The *diabetic* pentosuria is rare. This occurs in very severe cases of diabetes whose inability to burn sugars extends also to pentose (Kulz and Vogel found pentoses in the urines of 64 of 80 cases).

*Idiopathic* pentosuria is a quite different condition, first reported by Salkowski and Jastrowitz in two cases of suspected glycosuria. In 1902 the number had reached only five or possibly six (Brat's case<sup>74</sup>). Bendix later collected twelve cases and adds one. It is interesting that several were old morphia habitués, but the pentosuria continued after the habit was cured in one case not in another.

<sup>73</sup> See Hofmeister, *Lehrb. d. Physiol. Chem.*, 1899, p. 519.

<sup>74</sup> *Münch. med. Wochenschr.*, 1903.

Janeway (*Am. Jour. Med. Sc.*, Sept., 1906) collected twenty-two and added two. These two were brothers. It seems to be a chronic condition and without symptoms, the sugar being an accidental discovery. Pentose is not found in large amounts—in Salkowski's case it corresponded to from 0.07 to 0.15 per cent. of xylose, in Bendix's case, from 0.4 to 0.6 per cent. Bial<sup>75</sup> found that such cases could assimilate glucose normally, but pentose as well. The only explanation, therefore, that he can offer is that an excess of pentose is formed. Bial also found the assimilation limit for hexose and pentose normal.

These urines reduce copper, but not well, as if only a trace of glucose were present. The reduction does not come at once, but after cooling, and suddenly throughout all the urine; they do not ferment, the urine is but slightly dextrorotatory, and with Nylander's the precipitate is only a gray. It is seen that they resemble the urine of lactosuria. V. Jaksch found that diabetics excreted from 48.98 to 82.02 per cent. of the arabinoses of the food, and non-diabetics 1 to 46.65 per cent. Non-diabetics excrete from 54.8 to 18.7 per cent. of xylose, while diabetics only a trace. Of rhamnose from 63 to 55 per cent. was excreted by non-diabetics, and from 3 to 13 per cent. by diabetics.

Xylose is one of chief general importance. This is dextrorotatory. It forms osazones with a melting point of 159° to 160° C., reduces copper and Nylander's solutions, and gives an orange precipitate with Rübner's test; the furfurol reaction is positive; it does not ferment.

The arabinoses are somewhat different. These are dextrorotatory (104 to 105 per cent.), reduce somewhat better than xylose, form osazones, the melting point of which is from 157° to 158° C. Otherwise they are very similar to the above. The inactive arabinose is of particular interest, since this is the sugar found in the ideopathic cases.

TESTS.—The pentoses give the phloroglucin reaction (as do also lactose and galactose), but with pentose the color test is confirmed by its characteristic spectrum, which is also true of glycuronic acid, which gives exactly the same test. This test, according to Tollen, is as follows: To a few cubic centimetres of urine are added an equal part of HCl (sp. gr. 1.19), then from 25 to 30 mg. of phloroglucin, and the solution warmed until a red color appears. This solution is examined spectroscopically at once for a band in the green.

Salkowski recommends the following modification: Five to six cc. of fuming HCl are warmed and saturated with phloroglucin, leaving some undissolved. This solution is then halved. To the one test-tube is added 0.5 cc. of the suspected urine, and to the other 0.5 cc. of normal urine. Both test-tubes are then placed in a beaker of boiling water. If pentose be present, this tube will soon present a red color, which begins above and extends downward. The solution is exam-

<sup>75</sup> Verh. d. XIX Kongr. f. inn. Med.

ined spectroscopically. The test-tubes should be removed from the water-bath as soon as the red begins to appear.

To exclude glycuronic acid the osazone must be determined (Salkowski). Two hundred to 500 cc. of urine are placed in a beaker, and 2.5 gms. per 100 cc. of urine of phenylhydrazin dissolved in an excess of acetic acid added (or 3.5 gms. HCl phenylhydrazin with 1.5 parts of NaAc). This fluid is then warmed until boiling begins. It is allowed to stand from one to one and one-fourth hours in boiling water and then cooled. If pentose be present in any considerable amount, a large sediment of crystals will appear. As soon as the crystallization is complete the precipitate is then recrystallized from a hot, very dilute alcoholic solution, and again until the melting point is constant.

The orcin-HCl test is recommended as a more specific one, excluding the glycuronic acid compounds. To the urine is added an equal volume of concentrated HCl, and then a small amount of orcin. The solution is then heated. If pentose or glycuronic acid is present, the fluid becomes a reddish-blue color with a characteristic absorption spectrum. The urine should first be decolorized with animal charcoal. The reddish color may not be seen, or only very transitorily, a green color being obtained. The solution is cooled until only warm, and then shaken out with amyl alcohol. A green fluid with characteristic absorption bands is obtained.

Bial's last modification of the Salkowski-Blumenthal test<sup>76</sup> is as follows: A reagent (HCl 30 per cent., 500 cc.; orcin, 1 gm.; 10 per cent.  $\text{Fe}_2\text{Cl}_6$ , 25 drops) is used. Four or 5 cc. of this reagent are heated to boiling, then removed from the flame and the suspicious urine added drop by drop, but not exceeding 1 cc. A fine green color appears at once, or very soon, if pentose be present. This test, he says, is not given by the glycuronic acid compounds or any other body but pentose. He considers his several critics now answered.

If hexoses be present, these should not be fermented, since the pentoses, although unfermentable, will disappear during the process (attributed to the bacteria with the yeast). These sugars should be precipitated as osazones, and then separated.

*Method of Külz and Vogel.*—From 1.6 to 3.2 litres of urine are used. For each 100 gms. of glucose are added 200 gms. phenylhydrazin plus 100 gms. glacial acetic acid. The urine is then heated on a water-bath for an hour and a half, cooled, and filtered. The filtrate is again heated on the bath for one and a half hours and filtered. The combined precipitates are well washed with cold water and digested in water at 60° C., which dissolves the pentosazone. Glucosazone is dissolved only on heating to boiling. One litre of water per 100 gms. of sugar is used, and the digestion continued twelve hours. This is repeated fifteen times. The hot extracts are filtered, then allowed to cool, and the pentosazone will separate. This is repurified, using less water, till the melting point is constant.

<sup>76</sup> Deutsch. med. Wochenschr., July 2, 1903.



To separate the pentoses the alcoholic solution is polarized. The zylosazone shows a strong constant lævorotation, while arabinosazone immediately after formation is dextrorotatory and then optically negative.

Cases of pentosuria are exceedingly rare, perhaps because unsuspected and therefore overlooked. This is the only sugar which, with many of the glucose tests, promises much trouble. Suspicious cases of glycosuria, in which the tests are unsatisfactory, should always be further examined.

**Inosite.**—Inosite occurs in a great many plants and vegetables. It occurs rarely and in small amounts in the urine of nephritics and diabetics, and also in other cases of polyuria. Naunyn mentions a case with 18 to 20 gms. per day, but emphasizes the fact that inosite is not a sugar, and the case was probably diabetes insipidus. Hoppe-Seyler considers that it occurs in all normal urines. The method of isolation and detection is rather long. We give it, since in other conditions also inosite is interesting.

The urine should be evaporated to one-quarter its volume. The urine is then precipitated with baryta water. The precipitate is washed, decomposed with  $\text{H}_2\text{S}$ , and evaporated (albumin must first be removed). After decomposition with  $\text{H}_2\text{S}$  the filtrate is allowed to stand and the uric acid first filtered off. The filtrate is then concentrated to a syrup and treated boiling hot with two to three volumes of alcohol. A precipitate rapidly forms. This is cooled, ether is added, and the crystals will slowly appear. These are to be purified by decolorization and recrystallization.

**TESTS.**—The inosite is evaporated with nitric acid on a platinum-foil to dryness. To the residue are added a little ammonia and one drop of  $\text{CaCl}_2$ . It is then again evaporated to dryness and a fine rose-red residue obtained (Scherer's test). This succeeds only when the inosite is fairly pure.

*Seidel's Test.*—This is the same as the above except strontium acetate is added instead of  $\text{CaCl}_2$ , and a fine green colored solution with a violet precipitate appears. This test is positive when 0.3 mg. of inosite is used.

*Gallois Test.*—The inosite solution is evaporated almost to dryness, the residue moistened with a little mercuric nitrate. On drying the solution a yellow residue is obtained, which on high heat is of a fine red color, which disappears on cooling and reappears on warming.

**Glycogen (or Erythrodextrin).**—This has been found in the urine of diabetics after the sugar disappears or diminishes as a dextrin-like substance which browns on the addition of iodine. The urine reduces copper after long boiling. To detect, the sugar is evaporated to a syrup, and KOH and absolute alcohol added until a cloud due to the potassium salts is obtained. The fluid is then decanted, the precipitate is washed several times with absolute alcohol, dissolved in acetic acid,

and reprecipitated with absolute alcohol. This precipitate is warmed with the alcohol and dried. A white tasteless powder is obtained, soluble in water, which reduces copper slowly and browns on the addition of iodine.

**Animal Gum (Landwehr).**—This is said to occur normally in the urine. It seems to be a pentose, is slightly dextrorotatory, and not fermentable. With the copper test is obtained a precipitate which on boiling does not blacken. Alfthan found it much increased in diabetes mellitus (1.2 to 36.9 gms. per day, normally 0.1 to 0.2 gm.). It is probably not one, but a group of bodies precipitable by alcohol.

**Laiose** is a sugar not yet well isolated and the nature of which is uncertain, found by Leo<sup>77</sup> in the urine of some diabetics. It is lævorotatory, non-fermentable, with a salty taste and little reducing ability except after long boiling. It gives an oily compound with phenylhydrazin. It is not always present.

**Maltose** was present in two cases, but in small and variable amounts.

**Isomaltose.**—This sugar has been demonstrated in normal urine. Whether preformed or formed from glucose is uncertain, since the latter transformation is very easy. The osazone is in very fine crystals, with a melting point of 150° to 153° C. It does not ferment, or only very slowly, reduces copper and bismuth, and is dextrorotatory. It is to be demonstrated as a benzoate.

**Melituria.**—In the case of some malingerers it may be necessary to recognize this sugar.<sup>78</sup> In Brown's case the urine was of high specific gravity, gave a positive Fehling's test, but not quite typical, and but very few crystals with phenylhydrazin. It fermented but very slowly, and was dextrorotatory. There may, therefore, have been a trace of glucose present or some of the cane-sugar inverted in the acid urine. The urine may be concentrated, boiled with dilute HCl for from twenty to forty minutes, neutralized with sodium bicarbonate, after which the typical tests of glucose plus levulose (polarization therefore zero) may be obtained.

**Acetone.**—Acetone occurs in all normal urines, the maximum normal amount being about 10 mg. in twenty-four hours. It is much increased, according to V. Jaksch and others, in the following conditions:

(1) **Alimentary.** Acetone is increased whenever the carbohydrates of the diet are limited. It is always increased on a rich proteid diet in normal persons, and hence during hunger. It is also produced by a rich fat catabolism, but it requires about 150 gms. to influence the output.

<sup>77</sup> Virchow's Arch., 1887, vol. cvii. p. 108.

<sup>78</sup> Brown, Johns Hopkins Hosp. Bull., May, 1900, p. 101.

(2) Febrile acetonuria. This is the most common pathological occurrence. It may occur with any fever, even light, and has no clinical importance.

(3) Diabetes. In this condition it occurs in the largest amounts, and means an advanced or long-standing case. It usually also means a severe case, yet to this there are exceptions, and its presence need not necessarily mean an unfavorable prognosis. Yet its presence should be watched, as it is some criterion of the acidosis, and both therapy and diet will be in some degree determined by the depth of the test. More than 5 gms. may be excreted daily. Great rises may follow slight fevers; it is decreased by alkalies; it may be reduced by adding carbohydrate to the food, and yet in diabetes its output does not depend alone on the diet, although fat may yield some. In diabetes it tends to increase toward death and in coma. It is present also in the breath, giving it a fruity odor, 150 mg. even being excreted in one hour through the lungs.

(4) Carcinoma cases in which inanition is not yet present. (5) Cases of inanition and cachexia. (6) Psychoses and lesions of the central nervous system, especially those associated with starvation. (7) Autointoxication. (8) Digestive disturbances, especially gastric ulcer. (9) Chloroform narcosis, in which case it is due to the increased proteid catabolism. (10) Pregnancy with a dead foetus. (11) Certain poisons: *e.g.*, phloridzin. (12) Extirpation of the pancreas.

In most of these cases there is little doubt that its source is proteid. Others claim (Schwarz) that it may also be formed from fats. The immediate mother substance of the acetone is probably diacetic acid.

Acetone,  $\text{CH}_3\text{COCH}_3$ , is a thin, colorless fluid of a specific gravity of .814 (at  $0^\circ \text{C.}$ ), and a boiling point of  $56.5^\circ \text{C.}$ , with a quite characteristic odor.

TESTS.—As a general rule only the distillate of the urine should be tested for acetone. From 250 to 1000 c.c. of fresh urine are used and a little acid, preferably phosphoric, added to prevent foaming. V. Jaksch advises that it be distilled with steam, in which case no acid need be used. A good cooler should be used in quantitative work, although practically this is not necessary. Most of the acetone will pass over in the first 10 to 30 c.c. Since diacetic acid is split up to acetone the urines should first be made alkaline and carefully shaken out with ether which is alcohol-free, if it is desired (as it seldom is) to exclude this body. This ether extract may then be shaken out with water and the latter tested for the diacetic acid.

As a preliminary, *Legal's Test* is recommended, and yet this is satisfactory only when large amounts are present. A negative report has little value.

To the urine or its distillate are added a few drops of fresh concentrated sodium nitro-prusside solution, and then KOH or NaOH until very alkaline. A ruby-red color is obtained which changes rapidly to yellow. The test thus far is also given by creatinin, hence while the fluid is still red glacial acetic acid is added in excess. If acetone is present the red color will change to a purple-red, and later to violet. Creatinin does not give this color change, but a yellow changing to green and finally to blue. Paracresol gives a reddish yellow solution; acetic acid a clear rose color. In place of KOH, Le Noble and Lee used  $\text{NH}_4\text{OH}$  to exclude aldehyde.

*Gunning's Test* is very satisfactory. To the distillate is added tincture of iodine or Lugol's solution ( $\text{KI}$  1.8,  $\text{I}$  1.2,  $\text{H}_2\text{O}$ , q. s. ad 30), then ammonia until a deep black precipitate forms, which later gradually disappears, leaving a yellow sediment of iodoform. This sediment is recognized by its color, odor, and microscopically by the six-

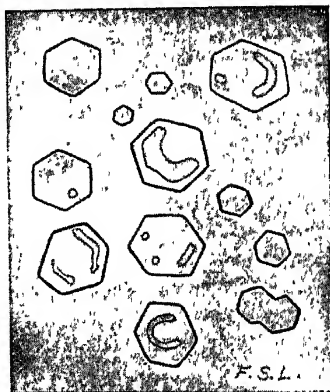


FIG. 28.—Iodoform crystals formed from the distillate of the urine of a case of diabetes.

sided tablets or stars. The sediment is seldom amorphous. In case of a trace it may be necessary to wait twenty-four hours. If necessary the sediment may be recrystallized from ether. This test may be applied to the urine directly, and is perhaps the safest clinical test for this purpose. Triple phosphate crystals are then also precipitated, and must be recognized. Gunning's test is less delicate than Lieben's, but is given by no other body than acetone. It shows 0.01 mg. per 1 c.c.

Denige's test,\* preferred by some to all others, is made as follows: To about half an inch of the distillate of the urine in a test-tube is added an equal amount of a solution of the subsulphate of mercury (mercuric oxide, 50; sulphuric acid, 200; water to 1000). This mixture is allowed to simmer about 5 minutes. When it cools there is deposited a white crystalline precipitate, which is distinctive in appearance, and is not soluble in dilute HCl.

\* See Taylor, Jour. of A.M.A., March 17, 1906, vol. 46, page 790.

Folin (*Jour. of Biol. Chem.*, May, 1907; *Jour. of A. M. A.*, May 2, 1908) has recently upset a few of the generally accepted ideas concerning acetone. He finds that the amount of acetone in the urine of even severe cases of diabetes is very small indeed; that the most of the substance estimated as acetone is diacetic acid; that some of the tests, *e.g.*, Legal's, supposed to show acetone are really very delicate tests of diacetic acid; and that the odor of the diabetics' breath and urine may be fruity, but this odor is not due to acetone.

QUANTITATIVE DETERMINATION OF ACETONE AND DIACETIC ACID—THE HUPPERT-MESSINGER METHOD.—This method determines the sum of acetone and the diacetic acid which is transformed to acetone by the distillation.

The solutions necessary are:

1. Acetic acid, 50 per cent.
2. N/10 iodine solution.
3. N/10 sodium thiosulphate solution.
4. A thin starch solution (1 gm. of starch dissolved in 500 cc. of boiling water).

To make up these solutions 24.8 gm. of crystallized sodium thiosulphate ( $\text{Na}_2\text{S}_2\text{O}_3 + 5\text{H}_2\text{O}$ ) are carefully weighed, dissolved in distilled water, and the solution made up accurately to one litre. Next 25 gm. of potassium iodide are dissolved in a little water, 12.7 gm. of iodine added, and the solution made up to about 900 or 950 cc. To standardize this solution 20 cc. of the thiosulphate solution are carefully measured into a small flask, a few drops of the starch solution added, and then the iodine solution added from a burette with glass stop-cock until the blue color just appears. This titration is repeated several times until the amount necessary for the end reaction is accurately determined, then to the iodine solution is added the necessary amount of water so that 20 cc. of this solution will exactly equal 20 cc. of the thiosulphate solution. This dilution should be confirmed by an additional titration.

Both of these fluids are to be kept in dark glass bottles with ground glass stoppers. The iodine solution must be restandardized frequently. One cubic centimetre of the thiosulphate solution equals 0.0127 gm. of iodine. The formula of the reaction is  $2\text{I} + 2\text{Na}_2\text{S}_2\text{O}_3 = 2\text{NaI} + \text{Na}_2\text{S}_4\text{O}_6$ . The first free iodine in excess will form the blue starch iodine compound.

The urine if alkaline is first just acidified with acetic acid. To 500 cc. of acid urine (if rich in acetone, *e.g.*, a febrile urine, 100 cc. or even less) is added 50 per cent. acetic acid, exactly 2 cc. per 100 cc. of urine. The urine is then distilled until only about one-tenth the volume remains. The flask receiving the distillate is tightly closed by a stopper with two perforations. Through one of these passes the tube from the distilling flask, and this tube reaches to the bottom of the flask and dips below the surface of water previously placed there; through the other passes a shorter tube connected with a bulb or Peligot U-tube filled with water, which acts as a safety bulb to prevent the loss of any of the very volatile acetone. This receiving flask is, during the distillation, surrounded by ice. When the urine is distilled

down to about one-tenth its original volume the distilling flask is disconnected before the heat is removed, else the distillate will "strike back." The tube of the cooler is then washed through with distilled water to wash into the receiving flask the last trace of distillate. The water in the safety bulb or U-tube is emptied into, and the tube washed and this water added to the distillate.

Some calcium carbonate is then added to the distillate and this well shaken. This is to remove any nitrous and formic acid which may have distilled over. The distillation is then repeated as before.

To this second distillate (to which is again added the water in the safety tube and the wash-water from both tubes) is added 1 cc. of dilute sulphuric acid (1:8 of water), and the distillation again repeated.

This final distillate is poured into a flask or measuring cylinder with ground glass stopper. (Or indeed this flask or cylinder may be used to receive the distillate during the distillation. It is of course protected as in the first distillation.) A large excess of carefully measured N/10 iodine solution is added, these fluids well shaken, and then an excess of strong nitrite-free NaOH or KOH added drop by drop. The flask is closed, shaken for one-quarter of a minute and then allowed to stand for five minutes. The vessel used must be so large that its contents will now not more than one-third fill it.

The stopper is then removed (and all fluid clinging to it washed back into the flask), the contents of the flask is then made acid with concentrated hydrochloric acid, and the excess of iodine determined by titration. The N/10 thiosulphate solution is added from a burette until the mixture is only slightly yellow, then a few cubic centimetres of the starch solution are added and the addition of thiosulphate solution continued until the blue color just disappears. If a little too much thiosulphate is added one adds a measured amount of iodine and continues the titration till the end reaction is reached. One cubic centimetre of the iodine solution equals 0.967 mg. of acetone.

The results of this method are from 4 to 8 per cent. too low. The final distillate must contain no phenol nor ammonia nor nitrous nor formic acids, for all of these but nitrous acid will cause the loss of some iodine while that will set iodine free. In this method the error from ammonia is prevented by the addition to the just-acid urine of 2 cc. of acetic acid per 100 cc. of urine. If a mineral acid, or 5 cc. of acetic per 100 cc. of urine were used none of the ammonia would reach the distillate but some phenol would. For this reason only 2 cc. of acetic per 100 cc. of urine are added and the trace of ammonia which does distil over is later removed by the third distillation after the addition of sulphuric acid. The addition of calcium carbonate to the first distillate will remove the nitrous and formic acids.

It is wise to add the alkali and the iodine solution to the final distillate without shaking much and to notice whether the fluid separating these two partial layers turns at all black. If so ammonia is present and the specimen should be thrown away. If none is indicated the fluid is shaken, etc.

An easier and fairly satisfactory determination may be made as follows:

From 50 to 250 cc. of urine are distilled until the great mass of water is passed over. To the end of the tube, which should have a good cooler, is attached a rubber tube, the end of which dips into water in the receiving flask. A little acid may be added to prevent foaming.

The distillate is poured into a graduated cylinder with a ground glass stopper, an excess (15 to 20 cc.) of NaOH added, and then 20 cc. of Lugol's, which is conveniently made three times the ordinary strength. A heavy black precipitate forms which soon clears, leaving a yellow sediment of iodoform crystals. After standing for some time, ten to fifteen minutes or more, about 40 to 50 cc. of ether are added and the fluid shaken out until the ether contains all of the iodoform. A reading is then made of the volume of ether, 10 cc. are removed in a graduated pipette and evaporated in the air in a weighed glass dish. This is then dried over sulphuric acid and the dish again weighed. The weight of the iodoform multiplied by 0.147 equals the weight of acetone represented in the number of cubic centimetres used. That of the whole ether extract may then be reckoned.

The above method gives the sum of the acetone and diacetic acid in terms of acetone. *Folin's method* (*The Jour. of Biol. Chem.*, May, 1907) is the only one which allows an estimation of the acetone alone. The acetone is separated from the urine by the apparatus which Folin has introduced for ammonia estimations (see page 130). Twenty-five cubic centimetres of urine are measured into the aërometer cylinder, from 0.2 to 0.3 gm. of oxalic acid or a few drops of 10 per cent. phosphoric acid, and 8 to 10 gm. of sodium chloride and a little petroleum are added. In the absorbing bottle has been placed water to which is added 40 per cent. potassium hydroxide solution (10 cc. per 150 cc. of the water) and an excess of the standardized iodine solution. The apparatus is then connected with a Chapman air pump and a fairly strong (yet not so strong as for an ammonia determination) air current drawn through for 20 or 25 minutes. Every trace of acetone will be removed from the urine and converted in the receiving bottle to iodoform. The contents of the receiving bottle are acidified with concentrated hydrochloric acid (10 cc. for each 10 cc. of alkali used) and the excess of iodine titrated with standard thiosulphate solution and iodine as in the Messinger method. The observer must be thoroughly acquainted with his air current and apparatus by repeated experiments with pure acetone.

**Diacetic Acid.** **Acetoacetic Acid**,  $\text{CH}_3\text{COCH}_2\text{COOH}$ .—Diacetic acid is the most important, from the clinical view-point, of the acetone

bodies, since it is much the easiest to test for, and would seem to predominate in amount. It should always be tested for in diabetes, since its presence in large amount is the best indication of a severe acidosis. The present idea is that all diacetic acid is derived from  $\beta$ -oxybutyric acid, and that all the acetone in the urine is in turn derived from diacetic acid. Only a trace, if any, is normal in the urine, and probably none if the person is on a mixed diet. It appears quite early in the urine of a person on starvation diet, or on a diet poor in carbohydrates, and promptly disappears if even a little carbohydrate be added to the diet. But the differences between individual cases in this particular are so great that its presence cannot be due to lack of carbohydrate in the diet alone. The statement usually made is that it occurs only when acetone is present in large amounts, but not always then. Nevertheless, the recent work of Folin would seem to prove that acetone is present but in traces, and that the most of the substance which we call acetone is diacetic acid. Since it is the mother-substance of acetone, its list of occurrences is practically the same as that of the latter. In most cases with much diacetic acid oxybutyric acid is probably also present, but the latter is more difficult to determine.

In most cases the cause of the appearance of diacetic acid in the urine is undernutrition or the failure of absorption, and hence occurs in all cachexia-producing diseases. It occurs in certain fevers, especially in the acute exanthemata of children during the eruptive stage, even in mild cases. Its occurrence and amount would seem to depend much on the nature of the infection, and the streptococcus infections seem especially favorable for its production. Its presence here is not at all limited to the febrile periods. In these cases it has no prognostic importance. It occurs with the greatest frequency in gastro-intestinal disturbances, even when mild, and is not due merely to the lack of carbohydrate, for it does not disappear when this is administered, as promptly as in health and on a pure proteid diet. In such cases it would seem to depend on abnormal fat catabolism. It is said to occur in especially large amounts in the urine of drunkards with gastro-intestinal disturbances. Rolleston and Tebbs<sup>80</sup> found it present in large amounts in 33 of 38 cases of gastric ulcer treated either by starvation or by rectal feeding, the test appearing in from two to twelve days, usually one to two days, after treatment begins, and disappearing in from one to fourteen days, usually the fifth, after mouth feeding is begun. Women especially showed the test; age and chronicity of disease seemed of no moment. In some cases the output of ammonia (index of acids) is as great as in diabetes

<sup>79</sup> See Neubauer and Vogel, p. 760.

<sup>80</sup> Brit. Med. Jour., 1904, vol. ii. p. 114.



(Golla). It may be found in the urine of normal men who have been for some days on a pure proteid diet, and in mental cases with loss of weight and inanition.<sup>81</sup>

*Gerhardt's Test.*—This very important test is best applied as follows: To 10 to 50 cc. of urine are added a few drops of  $\text{Fe}_2\text{Cl}_6$  solution, which must not be too acid. This is added as long as a precipitate forms, and then the urine filtered. To the filtrate is added still more  $\text{Fe}_2\text{Cl}_6$ . If diacetic acid be present, the urine takes on a Bordeaux-red color, cherry-red by transmitted, purple-red by reflected, light. The test indicates from 0.4 to 0.5 p.m. of diacetic acid. Cyanates, NaAc, salicylic acid, its allied bodies, salol, aspirin, diuretin, and certain other bodies also will give it. The colors obtained are not just the same, with strong solutions not at all similar, yet by adding various amounts of reagents, exactly the same color, it is said, can be produced, hence the test should always be controlled by boiling the weakly acid urine and repeating the test after the urine has been cooled. The red should be fainter, since the diacetic acid has in part been decomposed, but to boil half an hour will not break it all up. The urine may be acidified with  $\text{H}_2\text{SO}_4$ , shaken out with ether, this with water, and the  $\text{Fe}_2\text{Cl}_6$  added. A violet-red color is seen in the water layer. The color pales in from twenty-four to forty-eight hours on standing, a necessary part of the reaction to exclude other bodies. The urine should always be tested fresh.

Riegler's test has been much criticised, and its negative nature is unsatisfactory. In its latest modification<sup>82</sup> 15 cc. of urine are mixed with 2 cc. of 10 per cent Jodsäure and 3 cc. of chloroform and shaken. If diacetic acid be present, the chloroform remains colorless, otherwise it takes up a violet color.<sup>83</sup>

**$\beta$ -Oxybutyric Acid,  $\text{CH}_3\text{CHOHCH}_2\text{COOH}$ .**—This is the mother-substance of diacetic acid and hence of acetone. It may, therefore, be looked for when diacetic acid is present in large amounts, but not necessarily found, since it breaks up to diacetic acid and acetone so rapidly. Its list of occurrences is the same for acetone except it is found much more rarely. This acid occurs in the urine of non-diabetics; scurvy, severe infectious diseases, starving insane persons, but these are all in malnutrition. Gerhardt has shown that it is present in the urine of a normal man after some days on a pure proteid diet (about 9 gms. in twenty-four hours were found).<sup>84</sup> This acid is of extreme importance, since to its acid intoxication (a better term is alkali starvation) is attributed the coma. If it once begins, the tendency is for it to increase; often about 50 gms. a day are excreted, and

<sup>81</sup> See also Fletcher, Med. News, October 8, 1904.

<sup>82</sup> Zeitschr. f. klin. Med., 1904, Bd. 54.

<sup>83</sup> For criticism, see Voltolini, Zeitschr. f. klin. Med., 1903, Bd. 48, p. 336.

<sup>84</sup> Gerhardt and Schlesinger, Arch. f. exp. Path. u. Pharm., 1898.

in one of Naunyn's cases 100 gms. a day for a long time. Whether oxybutyric acid is toxic because of its acid nature alone, or has a specific toxicity is in doubt, the Strassburg school holding the former idea. Wilbur,<sup>85</sup> from animal experiments, injecting the neutralized acid, found the results similar to those of the free acid. He remarks that the alkaline treatment is not as satisfactory in coma as one would from theoretical grounds expect. Larger amounts are reported—even 188 gms. in twenty-four hours (Magnus-Levy); Külz, 225 gms.; and Joslin's case, 437 gms. in three days,—*i.e.*, 3 gms. per 1 K per day.

This acid is lævorotatory,— $[\alpha]_D = -24.12^\circ$ . Its presence, therefore, is possible when the percentage of sugar measured with the polariscope is less than that by titration. The presence of other lævorotatory bodies should always be considered, levulose, paired glycuronic acid compounds, albumin, etc.

**DETECTION.**—It is probably present if the fermented urine of a diabetic shows some lævorotatory body. It is quite surely present if the ether extract of urine first fermented, then acidified with phosphoric acid, and then extracted with ether, is lævorotatory.

The urine may be fermented, evaporated to a syrup, and an equal amount of concentrated  $H_2SO_4$  added. This is then distilled directly, and crotonic acid is obtained. The distillate cooled gives beautiful crystals with a melting point of  $72^\circ C$ . If these crystals do not readily form the distillate may be shaken out with ether, the ether evaporated, the residue washed with water and allowed to crystallize.

For two valuable unpublished methods I am indebted to Dr. Otis F. Black.

**BLACK'S QUALITATIVE TEST FOR  $\beta$ -OXYBUTYRIC ACID.**—The test depends on the oxidation of  $\beta$ -oxybutyric acid to acetacetic acid in the presence of ferric chloride, giving the well known color reaction. From 10 to 15 cc. of urine are concentrated in a small evaporating dish at a gentle heat to a few cubic centimetres, thus getting rid of the acetacetic acid, if present. The residue is acidified with one or two drops of concentrated hydrochloric acid and made to a thick paste with plaster of Paris. This is stirred and pulverized as it sets, with a blunt stirring rod, and the mass stirred up and decanted twice with ether. The ether is evaporated and the residue taken up with water and neutralized with barium carbonate. To the solution thus obtained in a test tube is added one or two drops of hydrogen peroxide and a few drops of a 5 per cent. solution of ferric chloride containing a trace of ferrous chloride. The characteristic wine red or violet color appears immediately, or on standing a few minutes, if  $\beta$ -oxybutyric acid is present.

**QUANTITATIVE DETERMINATION.**—The methods proposed for determining this acid are several. A good clinical method is the difference between polarization and titration. Both must be accurately done. This is the best method, and in all severe cases is well worth the time.<sup>86</sup> Others, Schwarz, *e.g.*, compare the result with Lohnstern's fermentation saccharometer with the polariscope. An easier method is the polarization of the fermented urine. The lævorotation must remain after the urine has been cleared with basic lead acetate and ammonia. There is no certainty that no acid is destroyed. The method used by Magnus-Levy who determined all of the inorganic acids and all the bases, and supposed the differ-

<sup>85</sup> Jour. Am. Med. Assoc., 1904, No. 17.

<sup>86</sup> See Pavy, Lancet, 1902, pp. 64-143, 207, 347.

ence to be due chiefly to this organic acid, is, we have very good reason to know, very laborious and does not seem to have proven particularly satisfactory.

Bergell<sup>87</sup> recommends the following: From 100 to 300 cc. of urine, fermented if glucose be present, are made weakly alkaline with sodium carbonate and evaporated on a bath to a syrup. This is then cooled and the residue rubbed up with phosphoric acid, cooling it meanwhile, then with 20 to 30 gms. of fused finely pulverized  $\text{CuSO}_4$  and 20 to 25 gms. of fine sand. This mass is well mixed. The dried mass is then put in a paper sac of the Soxhlet ether extraction apparatus and extracted with ether (which has been freed from water by the fused  $\text{CuSO}_4$ ) for one hour. (Black has devised a home-made ether-extraction apparatus which we believe is very superior to the usual form of Soxhlet apparatus, since any one can make it, and the substance to be extracted is in contact with hot ether.) It is then filtered and the  $\text{CuSO}_4$  washed out with dried ether. The ether is then distilled off, the residue taken up by 20 cc. of water, decolorized with animal charcoal, and polarized.

**BLACK'S QUANTITATIVE METHOD FOR  $\beta$ -OXYBUTYRIC ACID.**—One hundred cubic centimetres or more of urine are measured into an evaporating dish, made faintly alkaline with sodium carbonate, and boiled down to one-third or one-quarter of the volume, then concentrated further on the water-bath to about 10 cc.

The residue is cooled, acidified with a few drops of concentrated hydrochloric acid until it reddens litmus distinctly, and mixed with plaster of Paris to a thick paste.

The mixture on standing a few minutes begins to harden.

It is then stirred and broken up with a blunt glass rod, yielding a porous mass which is transferred to a Soxhlet apparatus and extracted with ether for two hours. The ether extract is evaporated, the residue taken up with water and treated with a little bone black if necessary, filtered, and made to known volume (25 cc. or less). The amount of  $\beta$ -oxybutyric acid is then determined with the polariscope.

Darmstadter<sup>88</sup> proposes the following method: To 100 cc. of urine is added enough soda to make it weakly alkaline. It is then evaporated on the water-bath almost to dryness, and the residue is washed by 150 to 200 cc. of 50 to 55 per cent.  $\text{H}_2\text{SO}_4$  into a 1-litre flask. This flask is then connected with the cooler of a distilling apparatus. Through the cork of the flask also enters a dropping funnel. The flask is just heated by a small flame until foaming ceases, then strongly, water added little by little from the funnel as it distils away, until 300 to 350 cc. of distillate have collected. This will take from two to two and one-half hours. The distillate is shaken out two to three times with ether, the ether residue heated a few minutes on a sand-bath at  $160^\circ \text{C.}$  to drive off the fatty acids, dissolved in 50 cc. of water, filtered, and the watery extract of crotonic acid titrated with tenth-normal  $\text{NaOH}$ , using phenolphthalein as indicator. One hundred cc. tenth-normal  $\text{NaOH} = 0.85$  gms. crotonic acid. The amount of crotonic acid multiplied by 121 equals amount of oxybutyric acid, hence 100 cc.  $\text{NaOH} = 1.0406$  gms. oxybutyric acid.

Boekelman and Bouma<sup>89</sup> propose the following simple method:

To 25 cc. of urine in a flask are added 25 cc. of 12 per cent.  $\text{NaOH}$ , then 25 cc. of benzoylchloride; the flask is corked and shaken hard under cold water for three minutes. The clear fluid is then pipetted off, filtered, and polarized. The benzoylchloride will clear the urine of carbohydrates, albumin, etc., leaving the acid the only laevorotatory body.

**Diabetes Mellitus.**—The urine in diabetes mellitus is, as a rule, increased in amount. This increase is often not marked unless there is

<sup>87</sup> Zeitschr. f. phys. Chem., 1901, xxxiii. 310.

<sup>88</sup> Zeitschr. f. phys. Chem., xxxvii. p. 355.

<sup>89</sup> Centralb. f. inn. Med., 1902, No. 25.

over 2 to 3 per cent. of sugar, beyond which point it is roughly proportional to the amount of glucose present. In severe cases, that is, with 5 per cent. of sugar or more, there may be from 4 to 5 litres of urine and even 10 litres, whereas one case is on record with 28 litres in twenty-four hours. On the other hand, there are cases with a high percentage of sugar and small amounts; two, for instance, reported by Naunyn, the one with 1400 cc., 9 per cent. of sugar, specific gravity 1040; a second, 2700 cc., 10.5 per cent. of sugar, specific gravity 1047. In other cases the reverse is true, but this is rare except in those cases following injury to the skull, in which during the day the specific gravity may be as low as 1003 with 1 per cent. of sugar (Naunyn's case); also in beginning chronic interstitial nephritis, and when the patient is very weak.

The urine has a high *specific gravity*, this being a condition with a high specific gravity and an increased amount of urine, but the specific gravity bears little relation to the latter. It is usually 1030 to 1040. Naunyn's maximum was 1060, and he mentions a case reported with 1074. With a specific gravity of 1030 there is almost always a diuresis.

The sugar is glucose, yet levulose and pentose and other carbohydrates are also present; in rare cases levulose alone. There is an increase also of the *unfermentable carbohydrates* (a minimum of 20 gms. instead of 1.6 gms.; normal maximum, 5 gms. per day).<sup>90</sup>

The urine is of a suggestive pale greenish-yellow color. It will ferment spontaneously, the fermentation resulting in the evolution of CO<sub>2</sub> and a sediment. This fermentation may occur in the bladder and a sugar-free urine be excreted; again, the fermentation may give no gas.

In testing the urine qualitatively for *glucose* it is important to choose the right specimen. In a severe case with sugar present at all hours this is not important, but in those mild cases with a very slight output, and for only a few hours after a rich carbohydrate meal, the sugar solution may in the whole twenty-four hours' specimen be so dilute that it escapes notice, while if the urine voided from two to four hours after a heavy carbohydrate meal be examined the test is clearly positive. It is well, therefore, to recommend to a suspected case a carbohydrate meal, preferably a breakfast, and examine the urine voided four to six hours later. It has been found that the maximum excretion is in the late forenoon, even when the ingestion of carbohydrates extends over the whole day. There is another maximum, somewhat less, in the late afternoon. Naunyn teaches that cases must be separated according to the percentage of the sugar excretion, the "intensity," and to the total amount of sugar excreted in a day,

<sup>90</sup> Edsall, Am. Jour. Med. Sci., 1901.

the "size." Cases can be compared in this way only when on a constant diet.

In cases whose sugar excretion is continuous a minimum occurs late at night and early in the morning, a maximum late in the afternoon and at about 6 P.M. In severe cases the variation is little marked and much more may be excreted during the night than during the day (note resemblance to the excretion of water and solids in nephritis). In the light cases the urine may be sugar-free during the night and even reach 3 per cent. during the day. Some cases will be sugar-free for months and then have periods of glycosuria. It is thus seen that the mild cases may easily be overlooked in case but one specimen is examined. The output is greater in hot than in cold weather, which is true also of the carbohydrate of normal urines.

The amount of sugar per day is often 800 gms., and in one case 1500 gms. in twenty-four hours. Such outputs occur only when the patient is on a liberal diet. On a strictly proteid diet cases excrete seldom over 100 gms., very rarely 200 gms.

As regards the relation between water and glucose excretion, diabetics respond to an increased intake more slowly than do normal people. In general it may be said that the water excretion depends upon the glucose, although this is not true of the day and night variations.

*Influence of Diet.*—The output is increased by a carbohydrate diet, especially dextrose and its polysaccharides. It is less influenced by levulose, lactose, and their polysaccharides. The starches of potato and oatmeal seem very well borne. If levulose, for instance, be fed, the sugar excreted is glucose, and yet a diabetic stands levulose quite well for a day or two, not longer. Lactose and cane-sugar are similarly treated. Fat causes no increase. Proteid causes a slight increase. In severe cases the output also varies with gastro-intestinal troubles, which are so common in diabetics and which may prevent absorption of sugar. Muscle work decreases it, and psychical influences, such as fright, mental strain, or worry, increase it much or bring a latent case to light. Hence in diabetics a peaceful mind is one of the essentials. Mendel and Lusk<sup>91</sup> found on a constant proteid-fat diet the constant ratio of glucose to nitrogen of 3.65:1, that is, the same as in the phloridzin diabetes of dogs.

They recommend this as a method of prognosis. The patient is put on a meat-fat diet (rich cream, meat, butter, and eggs), and the twenty-four hour urine of the second day is collected (the day ending to include the early morning urine). If N:glucose: : 1:3.65, it means the complete intolerance for carbohydrates and probably a quickly fatal outcome.

*Intensity of Glycosuria.*—On a rich carbohydrate diet the percent-

<sup>91</sup> Deutsches Arch. f. klin. Med., 1904, vol. lxxxi.

age rarely exceeds 6 to 8 per cent. Naunyn mentions a case of 11 per cent., while others mention a case with 20 per cent.

The effect of *acute infections* is particularly interesting, since in pneumonia, for instance, there is a remarkable diminution in sugar output with even an increased tolerance to carbohydrates. This is not due to the diet, and begins with the rise of temperature. The explanation is not known. On the other hand the sugar may be increased during the fever, or be present during an intermission, or be found after the fever, hence the statement that the complicating disease has been the cause of the diabetes.

Chronic diseases such as tuberculosis of the lungs, nervous diseases, circulatory disturbances with albuminuria, and nephritis tend to diminish the sugar output. As some diseases develop, and this is true of Bright's disease especially, the glycosuria wholly disappears, and they are reported as cured. This is not due alone to the diet, since the tolerance to carbohydrates is actually increased. Neither is it due to the kidneys, since the percentage in the blood does not increase. It may be due to the cachexia sometimes present.

The sugar output is subject to spontaneous fluctuations of considerable amount, even 100 per cent. These cannot be explained in any way except as variations in tolerance.

*Severity and Tolerance.*—A light case is, according to Naunyn, one which can eat daily 60 gms. of bread and remain sugar-free for a long time. By "paroxysmal tolerance" he means one that can stand considerable carbohydrate and be almost sugar-free, and yet which it is impossible to rid of that last trace. These are severer cases. The old rule that a light case was one which was sugar-free on a strict diet will not hold, since even a proteid diet is not sugar-free and severe cases may keep sugar-free for some time, and yet it be expensive for the body. There is a constant tendency for a large glycosuria to increase, and the greater the glucose output the weaker becomes the tolerance. The slight glycosurias tend to diminish. This tolerance suffers more from large amounts of glucose at one time than from the same amount in divided portions. The reverse is also true that tolerance is increased more by a brief sugar-free period than by a longer period of moderate output, hence in treating a diabetic the value of the "hunger day." If by a total fast the patient is sugar-free for twenty-four hours, on the following day he will often be able to stand without any glycosuria an amount of bread which previously would have caused a marked rise.

A case of transitory "diabetes" with acidosis is reported by Mann<sup>92</sup> which lasted sixteen days, then disappeared even though much sugar was consumed.

<sup>92</sup> Berl. klin. Wochenschr., 1904, No. 30.

The best routine method of determining the severity of a case of diabetes mellitus is that described by Janeway.\*

The patient is put on an accurately weighed test diet consisting wholly of proteins and fats, and having a fuel value of at least 35 calories of heat per kilo of the patient's weight. The diet he suggests, one adapted to American patients, is given below. If, however, the patient is a severe case and has been on a liberal diet, a sudden change to so strict a diet as the following would be too severe, so that 90 gms. of white bread are added, and this quantity is then gradually reduced.

#### STANDARD STRICT DIET.

Breakfast: Coffee with  $1\frac{1}{2}$  ounces cream. Two eggs cooked with  $\frac{1}{2}$  ounce butter. Three ounces ham.

Luncheon: Bouillon with 1 raw egg. Three ounces sirloin steak, chicken, or leg of lamb. One ounce bacon. Vegetable from list, 2 tablespoonfuls, with  $\frac{1}{2}$  ounce butter. Dessert made with 1 egg and  $1\frac{1}{2}$  ounces cream.† Six ounces wine, or 1 ounce whisky or brandy.

Afternoon tea with  $\frac{1}{2}$  ounce cream.

Dinner: Any clear soup. Three ounces fish (salmon, shad or mackerel), with  $\frac{1}{2}$  ounce butter. One-quarter pound roast pork, beef, mutton, turkey or lamb chops. Vegetables from list, 2 tablespoonfuls, with  $\frac{1}{2}$  ounce butter. Salad with  $\frac{1}{2}$  ounce oil in dressing. One ounce cheese, English, pineapple, Swiss or full cream. Six ounces wine, or 1 ounce whisky or brandy. Demitasse of coffee.

Protein=126 gms., 515 calories. Fat=222 gms., 2065 calories. Carbohydrate=15 gms., 60 calories. Alcohol=30 gms., 210 calories. A total of 2850 calories.

#### *Vegetables Allowed.*

Asparagus, beet greens, Brussels sprouts, cabbage, cauliflower, celery, chicory, cresses, cucumbers, egg plant, endive, lettuce, mushrooms, radishes, rhubarb, salsify, spinach, string-beans, tomatoes, vegetable marrow.

This diet is continued for one week. If while the patient is on this diet his urine becomes free from sugar, he is one of the milder cases; if the glycosuria continues after seven days of this diet he is one of the severe cases.

If the patient belongs in the group of milder cases, his tolerance

\* Am. Jour. of Med. Sci., March, 1909.

† The following recipes for desserts are suggested:

Baked custard: One egg,  $1\frac{1}{2}$  ounces of cream,  $2\frac{1}{2}$  ounces of water; two or three  $\frac{1}{2}$ -grain saccharine tablets, 8 drops of vanilla essence. Beat up well, pour into a buttered dish, grate a little nutmeg on top, and bake twenty minutes.

Coffee ice-cream:  $1\frac{1}{2}$  ounces of cream,  $1\frac{1}{2}$  ounces of water, 1 ounce of strong coffee, two or three  $\frac{1}{2}$ -grain saccharine tablets. Dissolve. Add one egg, well beaten. Mix in a saucepan and heat slowly with stirring until it thickens. Set aside until cool; then freeze.

is next determined by adding daily to the above strict diet weighed amounts of white bread in increasing quantities (the amount is usually increased 20 or 30 gms. each day), until sugar appears in the urine. The amount of bread given each day is equally distributed among the three meals. Tolerance is stated in terms of ounces or grams of white bread. For instance, if a patient's urine is free from sugar while he is on the above standard strict diet, and remains so on the day when twenty grams, and also when forty grams, of white bread are added to this diet, etc., but if on the fifth day, when 100 grams are added, sugar appears in the urine, this patient would be referred to as a mild case with a tolerance of 100 grams of white bread. A patient who for a long time has a tolerance of 60 grams or more is known technically as a mild case.

If, however, the glycosuria continues after seven days of strict dieting, the patient's tolerance is further tested by reducing the amount of protein of the strict diet. The urine should contain daily about 4 grams of urinary nitrogen, and the fuel value of the diet should remain unchanged.

Janeway suggests the following diet:

#### STANDARD DIET WITH RESTRICTED PROTEIN.

Breakfast: Coffee with  $1\frac{1}{2}$  ounces cream. Two eggs with  $\frac{1}{2}$  ounce butter. One ounce bacon.

Luncheon: Two eggs. One ounce bacon. Two ounces lamb chops (1), ham (2), beefsteak (3), chicken (4), or fish (5) broiled with  $\frac{1}{2}$  ounce butter. (Each day select meat with same number for luncheon and dinner.) Vegetable from list, 2 tablespoonfuls, with  $1\frac{1}{2}$  ounces butter. Dessert made with 1 egg and  $1\frac{1}{2}$  ounces cream. Six ounces wine or 1 ounce whisky or brandy.

Afternoon tea with  $\frac{1}{2}$  ounce cream.

Dinner: Any clear soup. One-quarter pound roast pork (5), beef (4), mutton (3), turkey (2), chicken (1), or lamb (1). (Each day select meat with same number for luncheon and dinner.) Vegetables from list, 2 tablespoonfuls, with  $\frac{1}{2}$  ounce butter. Salad with  $\frac{1}{2}$  ounce oil in dressing. One ounce cream cheese. Six ounces wine or 1 ounce whisky or brandy. Demitasse of coffee.

Protein = 82 gms., 334 calories. Fat = 215 gms., 2008 calories. Carbohydrate = 15 gms., 60 calories. Alcohol = 30 gms., 210 calories. A total of 2612 calories.

If the patient's urine becomes free from sugar while it contains not less than 14 grams of nitrogen, the patient has a moderately severe case of diabetes mellitus. But if the glycosuria continues on a carbohydrate free diet of which the protein also is so restricted that the urinary nitrogen falls below 14 grams the patients has a severe case. The most severe cases have a glycosuria when on a diet free from sugar and protein, and also when starving. Since the severity of a case depends not only on its glycosuria, but on its degree of acidosis, the amounts of acetone, diacetic acid, and ammonia



must be daily determined while the above dietetic experiments are being made.

*Coma and Acidosis.*—By “acidosis” Naunyn meant the production in the body by disturbed metabolism of acids in abnormal amounts sufficient sometimes to cause an acid intoxication, or, better expressed, an alkali starvation, resulting, it is believed, in the diabetic coma. The urinary symptom of acidosis is the appearance of large amounts of acetone or diacetic acid, and, in severe cases, oxybutyric acid, probably the mother-substance of the others. The production of these bodies is not characteristic of diabetic disturbances of metabolism, since a normal person on a sugar-free diet will in a few days produce them all. In diabetics there may be from 20 to 30 gms. of oxybutyric acid excreted daily for years. When an acidosis once begins the tendency is for it to increase. It is increased also by a rigid diet. This is the reason why the introduction of the strict dietary treatment of diabetes was followed by a great increase in the number of cases of coma. The severe cases should never be rigidly dieted. As coma comes on there is usually a sudden rise in these acid bodies, coma being preceded by days or even months with a daily output of 20 gms. or more of oxybutyric acid, but the output of 25 gms. indicates oncoming coma (Herter). The greatest increase follows the improvement in coma, for it is not the acid in the urine which causes the trouble, but the acid which has not been excreted. The presence of acidosis means usually a severe case, or at least an advanced case in which emaciation has begun, and yet the condition may exist for years. Patients with much acidosis die of coma unless from some intercurrent disease, and there is no case of true coma yet studied without a previous acidosis. The amount of these bodies is perhaps better estimated by the ammonia output than by their direct determination, since the symptoms are caused by a withdrawal of the body alkali which the ammonia protects. An increase of ammonia means the presence of at least 10 gms. of oxybutyric acid per day,—a marked increase, about 15 gms., 4 gms. of  $\text{NH}_3$  indicating 16 gms. of the acid (Herter). If there is a daily output of 2 gms. of ammonia and a positive test for diacetic acid one should avoid rigid dieting and should increase the alkali medication. Naunyn considers that over 3 gms. of ammonia per day means danger of coma. Coma was the terminal event of 18 of Naunyn’s 44 fatal cases, the most of them young persons from twenty-one to thirty years of age.

A sign, sometimes important, of coma, seen well in many cases and in one of ours, is the appearance of large numbers of granular casts giving a gross sediment. This may appear with the coma or give warning twenty-four hours in advance (Külz sign, page 282).

Among the other urinary symptoms in diabetes is an increase in

the outputs of the creatinin (even 2 gms. q. d.), uric acid, phosphoric acid, and sulphuric acid. In all of these conditions, however, the increase is due to the diet and not to the disease. Oxalic acid is also increased, especially as the sugar disappears (even 1.2 gms. per day). The animal gum of Landwehr is much increased.

Albumin is quite often present. In Naunyn's cases of pure diabetes 32 of 94 had albuminuria, 17 of whom showed occasional traces, 6 long-standing traces, and 10 much albumin. Ruling out those cases in which the albuminuria is due to complicating diseases, Naunyn considers that there is a certain relation between diabetes and albuminuria resulting from the effect of glycosuria on the kidney. On the other hand, it is interesting that as chronic nephritis develops, the glycosuria gradually disappears. This explains many so-called "cures." In some cases the glycosuria and albuminuria may alternate.

**Diabetes Insipidus.**—The cases from this clinic were recently reported by Fitcher in the Johns Hopkins Hospital Reports.

This is a very rare condition. Fitcher reported from this clinic but four cases, or 0.001 per cent. of admissions. It occurred particularly in young men. Jacobi, however, considers that fully 25 per cent. of the cases are in children under ten years of age. The cases may be grouped as *primary* or *idiopathic*, which have no known lesion, and the *secondary* or *symptomatic*. The latter cases are due to tumors of the brain especially, to cerebral trauma and hemorrhage, to cerebral lues and to basilar meningitis. It occurs also in certain diseases of the abdominal viscera and of the spinal cord. Polyuria is an occasional symptom of the psychoses, of hysteria, epilepsy, and chorea, some of which cases suggest strongly diabetes insipidus.

Polyuria is the only necessary symptom of the disease. From 20 to 40 litres may be voided in twenty-four hours, and in one case, 43 litres. In two cases of children the amount voided daily was almost equal to the body weight. The urine is pale, watery in color, faintly acid in reaction, and of exceedingly low specific gravity—from 1000 to 1005. (It is exceedingly important in this disease that the temperature correction in the specific gravity determination be carefully made.) In other cases there may be a polyuria with a normal specific gravity; *e.g.*, 6 litres and 1017. Albuminuria and cylindruria are absent, this absence indeed being the important point to rule out chronic interstitial nephritis.<sup>93</sup> Sugar is also absent, and yet there are cases of diabetes insipidus which will develop into diabetes mellitus, and *vice versa*. Brackett's case<sup>94</sup> began with sudden onset as polyuria following mental shock, and just before death, seven months later, the specific gravity rose from 1002 to 1006 to 1026 and considerable

<sup>93</sup> See Blaikie's case, *Lancet*, 1904.

<sup>94</sup> *Lancet*, 1899, No. 25.

sugar appeared. It is an interesting fact which several have noticed that some patients with diabetes insipidus will daily void an amount of urine which is greater than is the volume of the fluid they drink. The urine voided by one of Fitcher's cases, for instance, exceeded the amount of fluid he drank by from 400 to 6355 c.c. per day. This patient was carefully watched to avoid deception, the fluids he drank were accurately measured, the amount of water in the solid foods was calculated and added to that of the fluids drunk, and the urine was carefully collected and measured. This continued for a long time.

The urea may reach 80 gms. per day or more. This is due to the enormous appetite which these patients have; in other cases, however, it is diminished. The sodium chloride and phosphoric acid output is either normal or slightly increased.

Inosite is occasionally found. This occurs in normal persons with polyuria resulting in the drinking of large amounts of water, and hence has no importance, it probably being the inosite washed out from the muscles. The total carbohydrates are not increased (Edsall, Alfthan).

Many believe that a condition of bradyuria exists, that is, that the fluid ingested increases the urine output slowly and the increase lasts longer than normal.<sup>95</sup>

**Glycuronic Acid,  $\text{CHO}(\text{CHOH})_4\text{COOH}$ .**—Glycuronic acid is an intermediate product of glucose metabolism which is excreted only when protected from oxidation by conjugation with some body, as camphor, or with substances which arise in the body as indoxyl, skatoxyl, paracresol, phenol, or with certain nitrogenous substances, as uramidoglycuronic acid. Amounts less than 25 mg. per 100 c.c. of urine are excreted, but this amount depends on the quantity of bodies present with which it can conjugate and not at all on the amount of glycuronic acid formed. This acid crystallizes with phenylhydrazine in beautiful needles whose melting point is  $114^\circ$  to  $115^\circ$  C. Free acid does not occur in the urine. It has the reducing properties of glucose for Cu, Bi, and Ag, reducing copper as well as does glucose. It does not ferment. With HCl and phloroglucin or orcin it gives the same color tests as the pentoses even including their spectrum. It gives the furfurol test. While it is dextrorotary, it occurs only in paired compounds and these all are lævorotatory, and explain the normal lævorotation of the urine of from  $0.05^\circ$  to  $0.17^\circ$ . It is much increased after the ingestion of camphor which pairs with it, and also after chloral hydrate. The orcin reaction is the most convenient one to use (see page 192).

Its chief clinical importance is the fact that its paired acid compounds occur normally, that they reduce copper after somewhat pro-

<sup>95</sup> Pribrâm, Deutsches Arch. f. klin. Med., 1903.

longed boiling, and are lævorotatory. If, therefore, the sugar reaction is suggestive, the fermentation test negative, and the orcin test good, they may be suspected. Since this acid has been shown to be a product of the normal oxidation of glucose, it is increased in diabetes mellitus, some claiming that in very mild cases the unoxidized sugar is present only in this form. Edsall<sup>96</sup> showed that the excess of benzoyl esters in diabetes was not always due to an excess of glycuronic acid, but found an increase in the unfermentable carbohydrates. They are present in all intoxications. He suggested that their increase may be considered a protective measure of the body to combat these intoxications. Glycuronic acid is increased in a great variety of conditions. Edsall doubts that it can even be used to indicate a latent diabetes, while recently Fisher has given evidence that the pairing of glycuronic acid occurs before any oxidation of the dextrose molecule has occurred, which would rather destroy the theories concerning its importance in diabetes.

**Alkaptonuria.**—This very rare and interesting condition has of late attracted considerable interest. Its rarity is evident from the fact that but 40 cases (29 of them men) were reported up to 1902 (Garrod). Of these, only four were in America (Futcher), and the number has not much increased since attention was called to the abnormality.

It seems a congenital and life-long condition, although some cases are intermittent, and Mittelbach's patient is confident that his followed an injury.

Such cases occur without symptoms and are discovered accidentally, by the mother, for instance, finding the napkins of the infant darkly stained, or an insurance company refusing an applicant as a diabetic.

Most observers consider alkaptonuria as a variant of metabolism; that the body is unable to burn homogentisinic acid; and that it is comparable to glycosuria. Tyrosine while the mother-substance of some cannot explain all of it. It is a normal intermediate product of the breaking down of the albumin of food and of the organs.<sup>97</sup>

Alkaptonuria seems to be a family disease, 19 of 32 cases occurring in seven families, one family having 4 cases, but there is only one case of inheritance (Osler's case).<sup>98</sup> Garrod<sup>99</sup> finds that 60 per cent. of the cases are children of parents who are first cousins. Others think that it is due to an intestinal mycosis, a peculiar intestinal ferment, etc.

The amount of reducing substance excreted varies from about 3.2 to 6.9 gms. in twenty-four hours. It is of interest (said Garrod) that

<sup>96</sup> Univ. of Penn. Med. Bull., April, 1906.

<sup>97</sup> See Langstein and Meyer, *Deutsches Arch. f. klin. Med.*, 1903, vol. lxxviii. p. 161.

<sup>98</sup> *Lancet*, January 2, 1904; see Futcher, *New York Med. Journ.*, January, 1898.

<sup>99</sup> *Ibid.*, December 13, 1902.

the output is for different cases so constant, and that a person either excretes this amount or none. No traces, no gradual increase or decrease, are seen.

The amount excreted depends somewhat on the diet, since it is reduced to about one-half on hunger days, and is reduced a little by a vegetable diet.

Its period of greatest excretion was supposed to be from one to three hours (Mittelbach) after a heavy meal, a point in favor of its intestinal origin rather than due to abnormal metabolism, since the greatest output of the products of metabolism is from four to eight hours after a meal. Garrod,<sup>100</sup> however, found the latter true here, hence its origin in the tissues.

The urine when fresh is very acid, of normal color, but rapidly becomes dark, reddish-brown and syrupy from oxidation, especially if made alkaline. It gives the copper tests, but not Nylander's;  $\text{AgNO}_3$  is reduced in the cold, it does not polarize, is not fermented, and gives no crystals with phenylhydrazin.

Of the alkapton bodies, two at least have been isolated, homogentisinic and uroleucinic acids. V. Jaksch includes the glycosuric acid of Marshall, which, however, may for the most part be the first mentioned. Other bodies may be present. Urines supposed to be rich in pyrocatechin are for the most part really cases of alkaptonuria.

HOMOGENTISINIC ACID,  $\text{C}_6\text{H}_3(\text{OH})_2\text{CH}_2\text{COOH}$ , is the most important and in most cases the only alkapton body. It is to this that the characteristic reactions of the urine are due. Its mother-substance seems to be tyrosine, for this, if fed a patient, is excreted as this acid, especially if the tyrosine be given in small doses.

To *isolate*, the urine is made strongly acid with  $\text{H}_2\text{SO}_4$  (1 to 12), 75 cc. per 1 litre of urine. It is evaporated on a water-bath to one-fifth volume and shaken out four or five times with three volumes of ether. The ether is then distilled off, the residue dissolved in water (30 to 60 volumes), filtered, the solution heated to boiling and precipitated with 20 per cent. PbAc. This is quickly filtered while hot to separate the resinous brown precipitate. On standing the lead salts will slowly separate. These are decomposed by  $\text{H}_2\text{S}$ , and the filtrate carefully evaporated first on the water-bath and then in vacuo. The acid will crystallize out.

Garrod recommends that the urine be heated to boiling, and from 5 to 6 gms. of solid PbAc per 100 cc. of urine be added. When these are dissolved the urine is filtered, and the filtrate allowed to stand twenty-four hours in a cool place. The lead crystals are ground fine, suspended in water, decomposed with  $\text{H}_2\text{S}$ , filtered, evaporated first on the water-bath and then in vacuo to a syrup.

UROLEUCINIC ACID,  $\text{C}_6\text{H}_3(\text{OH})_2\text{C}_2\text{H}_3\text{OHCOOH}$  (?) has also been found in alkaptonuria. It is very similar to the above, with the reactions practically the same. It may be separated from it since it is precipitated by basic lead acetate. Garrod found none in the urine of those in whom years previously others had found it.

<sup>100</sup> Lancet, November 30, 1901.

Baumann's quantitative method:<sup>101</sup> 10 cc. of the urine are mixed in a flask with 10 cc. of 3 per cent. ammonia. To this mixture one adds at once several cubic centimetres of tenth-normal  $\text{AgNO}_3$ , shakes it a little, and allows it to stand five minutes. To the mixture are then added 5 drops of 10 per cent.  $\text{CaCl}_2$  and 10 drops of ammonium carbonate solution. After shaking, it is filtered. The brown-colored but entirely clear filtrate is tested with silver nitrate. If there at once occurs a strong precipitation of reduced silver, in the second test a larger amount, even twice as much silver solution is added to the mixture of 10 cc. of urine and 10 cc. of ammonia. As soon as one has determined approximately the amount of the silver solution necessary for oxidation, the end reaction may be determined with  $\text{HCl}$ , it being near when the deep brown fluid on the addition of  $\text{HCl}$  takes a light red color. The end reaction is reached when the filtrate from the silver precipitate on acidifying with dilute  $\text{HCl}$  gives a slightly visible cloudiness of  $\text{AgCl}$ . One can determine this point very sharply after repeating the determination from four to six times. If more than 8 cc. of the silver solution are necessary, on repeating the determination 20 cc. rather than 10 cc. of ammonia are to be used.

One gm. of the water-free homogentisinic acid is reduced with the above technique by a quantity of silver solution which contains 2.6 to 2.65 gms. of silver; that is, 240 to 254 cc. of the tenth-normal silver solution. Hence 1 cc. of the tenth-normal solution indicates 0.004124 gms. of the acid.

The method is rather approximate, having an average error of 6.1 per cent.

#### PROTEIDS IN THE URINE

**Albumin Tests.**—A urine which is to be tested for albumin should be both fresh and clear. If it cannot be examined very soon after it is voided, it should be protected from decomposition; otherwise its rapid changes in reaction may confuse some of the tests. If turbid, and the turbidity is not due to bacteria, it is best cleared by filtration through several thicknesses of paper. But when it is turbid bacteria usually are present, and then the urine is best cleared by filtration through infusorial earth, to which some prefer an asbestos filter or magnesia. Infusorial earth has this disadvantage, that it certainly removes some of the albumin. A concentrated urine should always be diluted, as the dilution will render albumin tests even more sensitive, rather than less so.

Hallauer's work<sup>102</sup> is interesting as showing the importance of this. If normal urine be concentrated by heat to one-half its volume and then serum albumin added, the heat and acetic acid test is even improved, but the heat and nitric acid test, Heller's, and the potassium ferrocyanide tests are negative. If the urine be previously evaporated to one-quarter its volume, none of these tests will show the albumin, yet all are positive if this concentrated albuminous urine be diluted to its original volume. The potassium ferrocyanide test is first to disappear, which it does when the specific gravity is about 1030. Urea and the neutral salts, especially phosphates, are the disturbing bodies.

The specimen should be chosen with care. If much albumin is present this is not necessary, but in very mild cases the albumin may

<sup>101</sup> Zeitschr. f. Physiol. Chem., 1892, vol. xvi, p. 270, or Hoppe-Seyler, Chemische Analyse, seventh edition, p. 460.

<sup>102</sup> Münch. med. Wochenschr., 1903, p. 1539.

be present during only a few hours of the day. In doubtful cases it is well to examine the urine passed in the afternoon, preferably after active exercise. Then there may be found a very distinct trace of albumin, which, had this voiding been mixed with several other albumin-free voidings of that same day, could not be detected.

*Heat and Acetic Acid.*—In this test coagulated albumin is precipitated. A test-tube is filled about to the top with filtered, clear urine, either neutral, or very faintly acid in reaction. Holding the tube by its lower end, one heats the upper layer of urine to the boiling-point. For careful work an alcohol flame is the best heat, since burning gas often forms a slight deposit on the glass, and this may be mistaken for a faint cloud in the urine. To see whether or not a precipitate has formed one holds the tube against a blank background. If turbidity appears in the upper part of the tube, it may be a cloud of albumin or of calcium phosphate and calcium carbonate. To rule out the latter, a few drops of 5 per cent. acetic acid are added until the urine is distinctly acid. The cloud of phosphates and carbonates will promptly disappear, the carbonates with effervescence. Instead of acetic acid Hammersten recommends hydrochloric acid, from 1 to 3 drops of a 25 per cent. solution per 10 c.c. of urine. After the addition of each drop of acid, the boiling should be repeated. The acid will render the cloud more distinct and more flocculent.

Even though the urine remains perfectly clear after boiling, the acid should still be added, since the urine may not be acid enough to permit coagulation. And, even though the boiled urine remains perfectly clear after the addition of acid, it may, nevertheless, contain a demonstrable amount of albumin, which will appear as a distinct cloud if the tube be allowed to cool for about fifteen minutes. It is said that some very acid albuminous urines are not clouded by boiling until a drop of alkali is added.

The most reliable of all the routine tests for albumin is, we believe, a slight modification of the test described above (see Hasting, *Medical Record*, July 7, 1906). To the urine in a test-tube are added one-fifth as much of a saturated aqueous solution of sodium chloride and from 3 to 5 drops of 50 per cent. acetic acid. After these are well mixed, the upper contents of the tube are heated to boiling in the manner described above. The sodium chloride renders the test more sensitive, and holds in solution all nucleo-albumin. Acetic acid in excess does not produce soluble acid albumin.

The more chronic the nephritis the whiter the albumin cloud. The more acute the browner the precipitate.

The other coagula which may appear are: The so-called "*nucleo-albumin*." This is precipitated in the cold by acetic acid. Two tests, the one in the boiled, and the other the unheated urine to which acetic acid has been added, may be compared to see if this body will explain all the precipitate. The urine gives a

better test for nucleo-albumin when diluted; the precipitate is soluble in excess of acid.

*Resinous acids* may cause error in case a great excess of acetic acid is added. This precipitate, which is soluble in alcohol, may be met with after the ingestion of different resinous bodies,—turpentine, benzoin, copaiba, balsam of Peru, Tolu, cubebs, etc.

*Heat and Nitric Acid.*—The urine is boiled as above, and then concentrated  $\text{HNO}_3$  added until strongly acid. The albumin precipitate is insoluble in fair excess, and hence the danger here is that too little rather than too much acid will be used. As a rule, about one-twentieth to one-tenth volumes are necessary. Hammarsten recommends one to two drops of 25 per cent. nitric acid per 1 cc. of urine. A flocculent precipitate indicates albumin. The phosphates are dissolved. The urine should be boiled before and after the addition of each drop of acid. If after adding the acid the urine be allowed to cool and a precipitate either appears or increases, it is of “albumose.” The “nucleo-albumin” is soluble in this excess of  $\text{HNO}_3$ . On cooling, uric acid may precipitate, but this should not confuse, since it is granular and colored. If only a trace of albumin be present, the addition of nitric acid may cause no precipitate unless the urine be rich in salts; on the other hand, a great excess of  $\text{HNO}_3$  may dissolve the trace which does appear, hence the boiling should be repeated after each drop. Here again the coagulation of a trace may not occur at once, and the tube should be allowed to stand some time, and then the coagulum may be found at the bottom.

By the above technique are excluded the “albumin normally present,” the “nucleo-albumin,” and “albumose” (Bence-Jones body). The urates may deceive in a concentrated urine, but this precipitate is never flocculent: also the resinous acids. Biliverdin and other pigments are said to confuse, but their precipitate is soluble in alcohol.

Another method much recommended is to render the urine strongly acid with a few drops of acetic acid. One-sixth volume of 30 per cent.  $\text{NaCl}$  (giving a 4 per cent. solution) is then added. A precipitate may appear in the cold, possibly of globulin, which is increased by heat. The urine is then heated as above. All albumins are precipitated, the “nucleo-albumin” cloud is slight or absent. Albumoses are shown which will dissolve on warming. The resinous acids may deceive. This test is less delicate than the preceding.

The heat and acid tests are very delicate, indicating 0.005 gm. per 100 cc. They should, however, always be confirmed, since the faintest trace is uncertain; by using them we lose the albumoses.

*Heller's Nitric Acid Test.*—This is a contact test between urine and nitric acid; if albumin be present there is formed a line of precipitate at the place of contact. It has the advantage that no flame is used. The albumin is precipitated as an acid albumin, which is insoluble in a fair excess of acid. Of all the mineral acids used, nitric acid requires less per molecule of albumin than the others. The pre-



cipitate is, however, soluble in a great excess of the acid. The test is very delicate, indicating, according to some, 0.007 per cent. and to others even 0.002 per cent. (Hammarsten). For this cold contact test is used a very large test-tube, or a wine-glass, or the albumoscope which is much to be recommended (a U-shaped tube with one arm very slender, allowing a beautiful layering of the two fluids [see Fig. 29], called also an horismascope).

Into the test-tube is poured about two inches of urine, and then one-third its volume of concentrated nitric acid is allowed to flow under very slowly, the tube being much inclined; or, the urine is made to flow on top of the nitric acid. Still better, the fluid added last is introduced from a pipette. The nitric acid must be colorless, con-

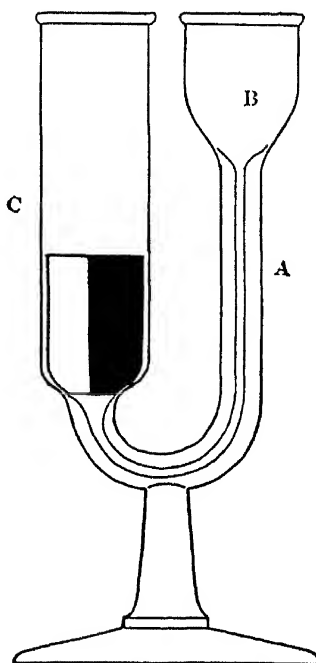


FIG. 29.—Horismascope. A, The arm of the U-shaped tube with fine bore; B, bulb in which  $\text{HNO}_3$  is poured after the tubes are filled with urine; C, wide-bore arm for urine, with background.

taining no nitrous acid since its effervescence with urea at the line of contact will disturb the ring and a faint one be lost; the same is true if much carbonate be present, as in an old urine. The line should be searched for against a dark background. In case the ring does not at once appear, one should wait, as it may show later. If no ring appears until three minutes the albumin is less than 0.003 per cent. This ring of acid albumin will appear exactly at the surface of contact; its thinness depends on the amount of albumin and also on the skill with which the urine has been superimposed.

The colored ring which always appears in concentrated urines should not deceive. This may be red or reddish-violet in color, but contains no precipitate.

The urate ring is present in all concentrated urines, and sometimes deceives. It is, however, above the line of separation, and when the test is well made is separated from it by a layer of clear urine from  $\frac{1}{2}$  to 1 cm. broad. This ring is broader than the albumin ring, less distinct, disappears on warming, and does not appear if the urine be diluted. To dilute the urine to about one-third volume does not interfere with the delicacy of the test nearly as much as is gained by the elimination of this ring.

One interesting case, the urine of which was sent to the clinical laboratory to demonstrate this fact, shows the value of diluting the urine. A consultant was called to see a case in which the attending physician had made a diagnosis of "albumosuria," the presence of Bence-Jones body in the urine, and had given a hopeless prognosis. It seems that he had tried this albumin test, obtained an abundant precipitate of urates which entirely clouded the urine and which disappeared on warming.

"*Nucleo-albumin.*"—This is present as an opalescent ring 0.5 to 1 cm. above the line of contact, sometimes extending down to it, and which disappears on slightly shaking the tube that the acid may be mixed with the urine, as this body is soluble in nitric acid. In the undiluted urine this ring appears somewhat later, is faint, does not resemble much the albumin ring, and in the case of a diluted urine appears even more rapidly and is clearer than in the concentrated.

*Resinous Acids.*—These may form a whitish ring above the line of contact and partly clear on warming. These bodies are soluble in ether, which should be added in great excess to prevent an emulsion. The precipitate is pipetted off for this examination. If suspected, the following test should be used. To from 8 to 10 cc. of urine are added 2 to 3 drops of HCl in the cold, which will precipitate these acids. On adding more HCl and heating a red color results. These resinous acids may also be extracted with ether from the urine made strongly acid with acetic acid.

The "albumoses" (Bence-Jones body) will also give a very heavy ring at the line of contact, which disappears on warming.

The bile acids will also give a precipitate in concentrated urines.

Urea nitrate may crystallize out. This line, however, which may form a solid crust between the two fluids, is so solid and so definitely crystalline that it will never deceive.

Hammarsten recommends that as a matter of routine all urines be diluted to a specific gravity of 1005. Dilution excludes all the above disturbing bodies except albumose and nucleo-albumin.

This test should never be trusted alone, but confirmed by another. Many workers recommend that it be used first.

*Potassium Ferrocyanide and Acetic Acid.*—A few drops of acetic acid are added until the urine is quite acid (containing about 2 per cent. of acetic),  $K_4FeCN_6$  (5 per cent.) is then added drop by drop, avoiding an excess. When the proper amount is added albumin is indicated by a cloud or flaky precipitate. If the observer be expert, this test is more accurate than Heller's. It takes, however, some experience, so much depends on the amount of reagents and the amount of salts present. The test is particularly valuable in quantitative work when it is desired to know if a solution has been rendered albumin-free.

The albumoses are also precipitated. "Nucleo-albumin" is precipitated, but also by acetic acid alone.

A hot urine must not be tested, nor any reagent used containing iron as Kieselguhr,<sup>108</sup> else clouds of inorganic precipitates are produced.

<sup>108</sup> Bardack, Zeitschr. f. inn. Med., 1902, No. 42.

*Tanret's Test.*—This reagent is made by dissolving 1.35 gms.  $\text{HgCl}_2$  in as little water as possible with 3.32 gms. KI (hence one molecule  $\text{HgCl}_2$  equals four molecules KI). To this solution are added 50 cc. of water and then 20 cc. of glacial acetic acid. This solution is added drop by drop to the urine, which will cloud should albumin be present, until the cloud just begins. It is exceedingly delicate. It indicates also “nucleo-albumin,” “peptone,” soluble on warming, alkaloids, and the albumoses. In French clinics we have seen this test used with the most satisfactory results.

*Spiegler's Test.*—Spiegler's test consisted originally of  $\text{HgCl}_2$ , 8 gms.; tartaric acid, 4 gms.; glycerin, 20 gms.; water, 200 cc. It has recently been modified by Jolles:  $\text{HgCl}_2$ , 10 gms.; succinic acid, 20 gms.; NaCl, 10 gms.; water, 500 cc.

This is the most delicate test of all. The urine is first filtered, rendered acid by a few drops of acetic acid to hold the carbonates in solution and to precipitate the nucleo-albumin, which is filtered off if present, since this also is precipitated, and then superimposed on the above reagent. A very sharp ring is produced by albumin. By means of it Spiegler claimed 1 : 50,000 could be detected, but as modified it is said there can be detected 1 : 150,000 to 350,000, the latter in the case of Jolles's modification. The advantage claimed for it is that it is definitely positive or negative, not suggestive. This detects also albumoses, but not deuterio-albumose. In case the urine be very dilute, that is, specific gravity 1005, the original test is of little value, hence NaCl is an ingredient of the modified solution. That the reagent will not mix with the urine, its specific gravity should be about 1060, hence a heavy acid is used and glycerin or saccharose added, and still more if necessary, as in a case of diabetes.

Various other tests have been proposed: Metaphosphoric acid in solid form, a piece the size of a pea being added to a test-tube of urine; picric acid is used also in the same way. Oliver has recommended papers saturated in the reagents because of the ease in carrying them around. Against all such tests should be said, however, that they are far inferior in delicacy to the above-mentioned tests, and hence fail where most needed.

There is a long list of very delicate tests recommended. In general it may be said that there is danger in these delicate tests, since it is granted a trace of albumin is normally present, and the test used should be only delicate enough to indicate a pathological amount. Almost any two tests which control the one or the other are good enough, providing the worker understand the shortcomings of each and be experienced in their use.

The order of delicacy of these tests is: Spiegler's, Tanret's, then heat and acid; next  $\text{K}_4\text{Fe}(\text{CN})_6$ ; next Heller's, then picric acid, and various others. This order, given by Huppert, is not accepted by some, who claim that they can with Heller's test properly performed in a wine-glass get more delicate results than with the heat. Senator recommends Heller's test, since it shows albumose. He advised

against the heat test, since by its use traces of albumin and rather large amounts of albumose are so often lost. We would recommend that no one test be used exclusively; that for routine work the heat and acid test be used after salt solution has been added to the urine, and that this be controlled by Heller's test.

QUANTITATIVE DETERMINATION OF ALBUMIN.—*Scherer's Method.*—About 500 c.c. of faintly acid urine are filtered. About 5 c.c. of this urine are then boiled in a test-tube and filtered. The filtrate is tested for albumin with acetic acid and potassium ferrocyanide. If this test shows that the filtrate is free from albumin, the acidity of the whole volume of urine is correct, and one should proceed at once with the determination. If, however, this test shows a trace of albumin in the filtrate, 2 or 3 drops of 50 per cent. acetic acid (and one drop when near the end) are added to the whole volume of urine, this is well stirred, and then 5 c.c. are removed and tested as before. This increasing (or decreasing it by a drop of strong NaOH solution if the proper point has been passed) of the acidity of the whole volume of urine is repeated until the filtrate of a sample that has been boiled is free from albumin. Two carefully measured quantities of the urine are now heated in a beaker, first on a water-bath and then over a free flame, until the precipitate is flocculent, and the supernatant fluid clear. The second beaker of urine serves as a control.

Cohnheim recommended the following modification, which we much prefer. To a carefully measured volume of urine is added one-tenth as much of a saturated solution of sodium chloride. One then proceeds exactly as above, but in the final calculation makes correction for the solution added. The addition of this salt renders complete precipitation of albumin a much easier matter and saves hours of work.

The proper quantities of urine to take for the analysis must be determined by judgment, since correct results are obtained only when the weight of the dried albumin precipitate lies between 0.1 and 0.3 gms. If it lies below 0.1 gm., the limit of error is too great; if above 0.3 gm., it is practically impossible to dry the precipitate to constant weight.

If the urine is rich in albumin, it should be properly diluted with salt solution; if very rich, the best results are obtained by pouring, drop by drop, a small, accurately measured amount of the urine into a beaker of boiling, half-saturated salt solution.

When coagulation is completed by boiling the urine over the free flame, the urine is filtered through a dried and weighed filter paper, and the precipitate is washed free from chlorine (a few drops of filtrate are tested at frequent intervals with  $\text{AgNO}_3$  solution) with

hot water, then washed with alcohol, and finally washed with ether. The paper containing the precipitate is then placed in a weighed weighing glass (with accurately fitting cover) and put in a drying oven the temperature of which is held at  $110^{\circ}$  C. The weighing glass should rest on a sheet of asbestos; the bulb of the thermometer of the oven should hang at the level of the weighing glass. At intervals of about one hour each glass is removed from the oven with a pair of tongs and cooled in a desiccator. The cover is then inserted tightly, and the glass is weighed. This is repeated until weight is constant. Even with most careful work the result and its control may differ even one per cent.

Salkowski<sup>104</sup> advises that when much albumin is present a small, accurately measured volume of urine be mixed with from 10 to 20 volumes of 95 per cent. alcohol, and that the mixture be brought to the boiling point on the water-bath. It is then cooled, the supernatant fluid is decanted, and the precipitate is washed with hot water, filtered, washed as above, then placed in a weighed platinum crucible and brought to a constant weight. It is finally burned, and the weight of the ash is determined and subtracted from that of the precipitate.

*Esbach's Tubes.*—An Esbach tube (see Fig. 30) is filled to the U with urine, and to the R with the reagent. The tube is corked, reversed slowly twelve times, and then left standing erect in a tube rack for just 24 hours. At the end of that time the height of the precipitate is noted. The figures on the scale indicate grams of albumin per one litre of urine.

Esbach's reagent (picric acid, 10 gms.; citric acid, 20 gms.; water, sufficient to make 1 litre) has not given satisfactory results and has been replaced by Tsuchiya's reagent.

Phosphotungstic acid,  
Concentrated HCl.,  
Ethyl alcohol, q.s. ad

1.5 gm.  
5 c.c.  
100 c.c.



FIG. 30.—Esbach's albuminometer.

Tsuchiya's method is quite accurate enough for clinical work (see Mattice, Arch. Int. Med., March, 1910, vol. v, page 313).

An approximate estimation can be based on Heller's test, made in a "Collamore wine-glass" half filled with urine, then underlaid

<sup>104</sup> Berl. klin. Wochenschr., March 3, 1902.

with approximately one-third its volume of nitric acid. By "slightest possible trace" is meant the smallest amount which can be detected as a haze under most favorable conditions (black background, etc.); "very slight trace" means slightly more; a "slight trace" can be seen without a black background and also from above, although the bottom of the glass is distinctly seen; a "large trace" (about 0.1 per cent.) is a clearly seen ring but not flocculent, quite dense but not opaque when seen from above; through the ring made by  $\frac{1}{8}$  per cent. the bottom of the glass cannot be seen, but a faint ray of light is transmitted; 0.25 per cent. gives a zone quite flocculent from the side and opaque from above; 0.5 per cent. and above, the ring is very dense and flocculent. Above this one cannot go by this method. The width of the ring is not so important (condensed from Ogden, "Clinical Examination of the Urine").

Rössler used Jolles's test in a similar way, but depended more on the thickness of the ring.<sup>106</sup>

*Centrifuge Method.*—Purdy has recommended that in graduated centrifuge tubes be mixed 10 cc. of filtered urine, 3.5 cc. of 10 per cent.  $K_4FeCN_6$ , and 1.5 cc. of acetic acid. The urine is then centrifugalized at a uniform speed of 1500 revolutions per minute in a centrifuge the arm of which is of such length that the distance from the centre of rotation to the tip of the tube is  $7\frac{3}{4}$  inches. It is centrifugalized three times, five minutes each time;  $\frac{1}{10}$  precipitate indicates  $\frac{1}{60}$  per cent. by weight of albumin. In his recent edition he gives a table with the equivalents of the readings. This test, satisfactory as it may seem, has not given very good results in our hands, although better than the Esbach tube. It is an interesting fact that two of the makers of the Purdy centrifuge were unable to supply us with an arm which conformed to his specifications as regards length, hence one must be made to order. It is difficult also to obtain graduated tubes with the sharp point as he represents them. We have found it no easy matter to keep a centrifuge running uniformly at this rate unless one stands over it and watches the taxometer during the entire time. The exact time the urine is centrifugalized is of great importance.

**Roberts and Stolnikow's Method.**—This method is based on the observation that with Heller's test a ring appearing from two and one-half to three minutes after the test is made indicates an albuminuria of 0.003 per cent. Diluted urines are therefore tested until a dilution is obtained in which the ring appears in this time. The test should be performed very carefully. The sides of the tube should not be wet with the nitric acid, and the urine should be added slowly from a pipette.

This determines at the same time the "nucleo-albumin" and resinous acids.

<sup>106</sup> Deutsches med. Wochenschr., 1903, No. 19, p. 335.

In the *Lohnstein method* the specific gravity of the urine is determined before and after the albumin is removed by heat and filtration. The difference multiplied by an experimental coefficient gives the albumin per cent.

It is often necessary to *remove albumin* from the urine before continuing with other quantitative work. To do this Hofmeister's method is the best. To the urine an excess (10 cc.) of sodium acetate, 40 per cent., and concentrated  $\text{Fe}_2\text{Cl}_6$  are added, until the whole is of a red color. The urine is then neutralized or very faintly acidified and then boiled. The precipitate of basic ferric acetate will carry down with it all of the albumin and leave the solution albumin- and iron-free, and this filters beautifully. This method cannot be used if glucose be present, since some ferric oxide remains in solution.

For practical purposes it is sufficient to boil and to add acetic acid until the precipitate is flocculent and the filtrate clear. The filtrate may be further tested as in the quantitative work. The urine should then be restored to the original volume.

**Proteids Present.**—By ALBUMINURIA is meant the presence in the urine of a coagulable albumin which has escaped through the cortex of the kidney (for false albuminuria, see page 230). Nearly always there are present serum albumin, "serum globulin," and the so-called "nucleo-albumin."

The ALBUMIN QUOTIENT is the amount of serum albumin divided by the amount of "serum globulin" present (Hoffmann). This quotient varies considerably in various cases, and in the same cases during various stages. In some cases serum albumin alone has been found. This was true of one case of cancer of the stomach, and of certain cases of nephritis during, however, limited periods. Globulin alone has been found in one case of acute nephritis, in the case of one woman during the puerperium, and in one case of leukæmia. (For "nucleo-albumin," see page 225.)

SERUM ALBUMIN is present normally even as much as from 22 to 78 mg. per 1 litre (Mörner). It is soluble in water, coagulated by heat in acid solution at a temperature varying from  $56^\circ$  to  $81^\circ$  C., depending on the amount of salts present, especially the phosphates, also the urea, and lastly on its own concentration; it is coagulated by absolute alcohol, which coagulum is soluble in water unless it has been in contact with the alcohol for a long time. This is rendered even more insoluble by weaker than by stronger alcohol. The solubility of this coagulum should be borne in mind by all doing quantitative work with this precipitate. Serum albumin is lævorotatory,  $[\alpha]_D = -62.6^\circ$ . The albumin plus alkali gives a soluble body which, when united with a base, forms an albuminate much less soluble in water than is albumin, and which will therefore give a spontaneous precipitate of albumin in a concentrated urine.

With mineral acids the acid albumin is quite insoluble until a large excess is added; in the case of acetic, however, a very slight over-acidity will dissolve the precipitate.

SERUM GLOBULIN is a term including several different bodies, among them pseudoglobulin, euglobulin, and fibrinoglobulin of the Hofmeister school, the reactions of which are rather different, but all of which exist in the blood-plasma. Euglobulin and fibrinoglobulin (fibrinogen) are probably always present normally in the urine. They are increased in the mildest forms of albuminuria (the so-called physiological and cyclic cases). In the severer cases albumin is present as well.<sup>107</sup>

The limits of precipitation by a saturated  $(\text{NH}_4)_2\text{SO}_4$  solution are the following, expressed in number of cubic centimetres of the saturated ammonium sulphate solution necessary to add to the urine, the amount of mixture to be in all cases 10 cc. Pseudoglobulin, 3.4 to 4.6; euglobulin, 2.8 to 3.3; fibrinoglobulin, 2.2 to 2.9.

Pseudoglobulin is not precipitated by acetic acid alone. Euglobulin occurs in almost all exudates and transudates, and in many urines, perhaps all. It is precipitated by acetic acid sometimes in the undiluted urine, but usually one must dilute two or three times. The acetic acid must be carefully added, since the precipitate is partly or wholly soluble in excess.

SERUM GLOBULIN (the group) is present in amounts varying from 8 to 60 per cent. of the total proteid; very rarely only a trace is present. In the blood the ratio to albumin is as 1 : 1.5. Its great increase over the albumin cannot in all cases be explained by its greater diffusibility, since euglobulin, which is constantly present, is less diffusible. The quotient (see page 223) varies in nephritis, the globulin being the variable factor. Oswald considers that an output of euglobulin is the mildest form of albuminuria, and that this is precipitated by acetic acid in the cold. This body is present in largest amounts in parenchymatous lesions. (See also Calvo et al.) As the nephritis improves the relative amount of globulin diminishes, and increases with each acute exacerbation. "In cases of contracted kidney and in chronic passive congestion with nephritis the quotient is the higher, from 2.8 to 5.3, but in amyloid disease the quotient may be lower than 1. In acute nephritis it may be very low. It is low but not so markedly so in the albuminuria of pneumonia, but the reverse is true of typhoid fever.

The globulins are insoluble in water, and in the urine are held in solution by the salts. If, therefore, to a beaker of distilled water a drop or so of urine be added a distinct cloud is seen. They are also

<sup>107</sup> Oswald, Münch. med. Wochenschr., 1904, No. 15.



detected by diluting the urine till the specific gravity be about 1002, then adding one drop of acetic acid.

TEST.—The phosphates are precipitated by rendering the urine alkaline with ammonia, and are then filtered off. An equal volume of cool saturated ammonium sulphate is then added to the filtrate, which will perfectly precipitate globulin in neutral solution, the mixture allowed to stand one hour, and filtered. The precipitate is washed with half-saturated ammonium sulphate until the filtrate is albumin-free. Albumose and “nucleo-albumin” are also precipitated. The precipitate of ammonium urate is to be avoided, but this comes later and does not look the same. Serum albumin is not precipitated until total saturation. The precipitate is dissolved in a little water, heated on a water-bath, which coagulates the globulin and fibrinogen and albumose. It is then filtered, the precipitate washed with water and digested on a water-bath with 1 per cent. soda. It is then filtered and neutralized carefully with acetic acid. The precipitate is of globulin and fibrinogen. Albumose would not be precipitated.

TO DETERMINE GLOBULIN QUANTITATIVELY the filtered urine is rendered neutral with ammonia, and to it is then added an equal volume of saturated ammonium sulphate solution. The mixture, well stirred, is allowed to stand for some hours. It is then filtered through a dried and weighed filter, and the precipitate washed with half-saturated ammonium sulphate until chlorine-free. This filtration is a slow process. The funnel and all are then placed in the thermostat and dried for half an hour at 110° C. The ammonium sulphate is then washed out with hot water, the precipitate dried with alcohol, ether, and then at 110° C. to constant weight. In this case also the amount of urine used should be such that the weight of precipitate could not exceed 0.3 gm.

**Euglobulin, Nucleo-Albumin, Mucin, Mörner's Body.**—In the urine in a great variety of cases occurs a proteid precipitated in the cold by acetic acid, giving an opalescence or true precipitate especially if a diluted urine be tested. It is difficultly soluble in an excess of acetic acid; the resinous acids should be excluded by the HCl test. With Heller's test the ring is not at the line of separation, but from 0.5 to 1 cm. above it. Both rings, that of albumin and this, may be present, in fact the best “nucleo-albumin” rings are seen in nephritis. It may, however, extend down to the acid. The urate ring should be excluded by testing a diluted urine, which makes the “nucleo-albumin” ring even more distinct. It coagulates at about 56° C., and we have known the diagnosis of albumosuria to be made from this point. The test is always improved if a diluted urine be used, and, in fact, can be obtained in probably every normal urine if the salts be removed by dialysis. It is first seen near the acid, then, as this diffuses upward,

the ring travels upward until all this proteid in the urine has been precipitated and then dissolved.

It is this body which first led to the belief that proteids were normal constituents of the urine. Two other and contrary views were held, one that it was mucus, the other that it was nucleo-albumin, hence the condition was not a true albuminuria; now many believe that it is globulin or a compound of serum albumin, hence is a true abuminuria. A precipitate on the addition of acetic acid occurs in the urine in a great number of conditions, so many that it is hard to classify them, and each writer has done so on the basis of his idea of its nature. Excluding the vesical cases, in which it probably is mucus, it is increased in the new-born; in adults after severe exercise, nephritis, and various acute diseases, especially those affecting the kidneys; fevers, especially pneumonia and typhoid (erysipelas, pleurisy, relapsing fever, meningitis). Its increase in leukæmia, reported first by Fr. Müller, is of interest in connection with the idea that it arose from the nuclei of the leucocytes.

Obermayer found it in 32 cases of jaundice, the amount depending on the intensity of jaundice and ceasing with it. He found it present in scarlet fever in small amounts, diphtheria in the greatest amounts of all, in connection with albuminuria after poisons affecting the kidneys (pyrogallie acid, corrosive sublimate, etc.), in acute yellow atrophy, and after compression of the thorax.

In true nephritis it may precede the true albuminuria and also succeed it, and remain when, by severe dieting, etc., this intermits. Madsen considers it a good test of the earliest irritation of the kidneys. Euglobulin and fibrinogen are said to be the chief proteids in amyloid kidney.

In orthostatic albuminuria this may be the only proteid present, or with albumin and pseudoglobulin. In febrile albuminuria it may exceed in amount the serum albumin. It is present in but traces in chronic interstitial nephritis. When the blood-supply of the kidney of animals is cut off, this body in abundance is excreted, sometimes with albumin, sometimes not, and the same is true in partial suffocation.

PURE MUCUS is present in the normal urine in traces (4.5 gms. in 260 litres). This may be found in two portions,—an insoluble which gives the nubecula, and a soluble portion precipitated by acetic acid, which is only a very small fraction of the whole. Mucus would be expected since the urinary passages are lined with mucous membrane, and hence the urine will gather a little of its secretion as it passes down to the bladder. This mucin is much increased in catarrhal conditions of the urinary tract, and is added to the urine as a gelatinous precipitate as this passes over the mucosa. It is soluble in ammonia, precipitated by acetic acid, and soluble in excess; from it a reducing

body may be split off. It does not contain nuclein, nor chondroitin, hence it is a mucin, but the absence of the slimy character of the precipitate with acetic acid gives it the term "mucoid." It resembles ovomucoid of the hen's egg (Mörner). In a recent case of prostatitis we found that the acetic acid precipitate was 0.066 gm. per 100 cc. of urine. The urine is precipitated carefully with acetic acid, and repeatedly filtered through a weighed filter till the filtrate is clear. The precipitate is then washed with cold water acidulated with acetic acid, dried, and weighed. Another method giving slightly lower results is the following: A small amount (0.5 gm.) of Kieselguhr is dried at 110° C. to constant weight, mixed with the urine, and dried with the paper and precipitate. It was much more rapid than the preceding. There are, however, other interesting and rare cases of true mucinuria analogous to mucous colitis and fibrinous bronchitis with casts 1 to 10 cm. long and 3 to 4 mm. thick in the urine. Such was v. Jaksch's case of "ureteritis membranacea" in which a spiral cast of the ureter of mucus and fibrin was voided; in Frank's case it was a cast of the pelvis and upper ureter. He named the condition "pyelitis productiva." Four cases are on record. In the above cases the symptoms of the expulsion of the casts resembled those of renal colic.

From the study of many cases of jaundice Obermayer decided that the body was nucleo-albumin, and hence all precipitates with acetic acid were considered nucleoproteid.

NUCLEO-ALBUMIN also may occur, but it is not the body that usually goes under that name, and it never occurs normally. Its presence has been claimed as due to the breaking down of the cells of the urinary tubules. The kidney is an organ very rich in cells and the disintegration of these would certainly set free a certain amount of nucleo-albumin. This may explain the nucleo-albuminuria of acute nephritis, the condition in which this proteid is present most constantly and in greatest amounts, also the nucleo-albuminuria following the ingestions of poisons which affect the kidneys, and that due to disturbances of renal circulation. Its origin in cases of jaundice is the bile. Nucleo-albumin is said by some to be present in the blood, and it is possible that a certain amount reaches the urine from this source. It may come from catarrhal conditions of the urinary tract with desquamation of the superficial cells of the mucosa. Such is true in cystitis or pyelitis. In the case of women the genital tract is to be excluded as a source.

It will be seen from the above list that the occurrence of nucleo-albumin is claimed for all cases in which theoretically nucleoproteid could occur; but in some of these conditions a true albuminuria occurs, and in others in which nucleo-albumin should be present in large

amounts (urines containing abundant pus and epithelial cells) it is hard to get any precipitate at all on adding acetic acid.

It is very clear, to one reading reports of cases, that in very few has the crucial test been applied, the proof that it is a phosphorus-containing body which acetic acid precipitated, and positive results would have to be scrutinized carefully, since it would be very easy for phosphorus to be an admixture from the urine.

Again, the "salting out" points with ammonium sulphate do not quite agree with those of true nucleo-albumin from breaking down tissue. Matsumoto gives as limits of its precipitation, minimal 0.1 to 0.8, maximal 1.6 to 2.2.<sup>108</sup>

To prove the body nucleo-albumin it should be insoluble in acetic acid, precipitated by  $MgSO_4$ , when boiled with dilute mineral acids give off no reducing substance, and on peptic digestion should give nuclein and contain phosphorus, but the last two tests it is almost impossible to apply to the urine.

MÖRNER'S BODY.—Work which seemed very convincing and which is now often quoted is that of Mörner.<sup>109</sup> According to him most of the so-called nucleo-albumin is a compound of true serum albumin with an albumin-precipitating body which is formed on the addition of acetic acid. Mörner, by dialyzing large amounts of urine and adding 1 to 2 parts per thousand of acetic acid, and then shaking with chloroform, obtained a precipitate which much resembled nucleo-albumin. This occurs on an average of 41 mg. (22 to 78) per litre of urine. Further investigation showed this to be a precipitate of serum albumin with chondroitin-sulphuric acid, which was always present and the most important, nucleinic acid, which is sometimes present in traces, and taurocholic acid, which is also sometimes present in traces, but which in the case of jaundiced urine may exceed the others in amount. Since there are these three possible combinations the precipitates will differ. If after removing this precipitate a little albumin be added to the urine, a second precipitate results of about 54 mg. per litre, showing that these albumin-precipitating bodies are in excess. Since normally in excess, any increase of precipitate would mean an increased excretion of albumin. The greater the predominance of these precipitating bodies, however, the more does the precipitate resemble nucleo-albumin. This union probably occurs after the addition of acetic acid. According to the relation between them the precipitate will resemble nucleo-albumin or serum albumin. If the albumin predominates, it may give its own proper tests. These bodies, and their relation, will explain the old statements, based on the common experience, that "a true albuminuria is sometimes preceded

<sup>108</sup> Matsumoto, *Deutsches Arch. f. klin. Med.*, 1903, vol. lxxv. p. 398.

<sup>109</sup> Skand., *Arch. f. Phys.*, vol. vi. p. 332, 1895.

by the excretion of a body precipitated by acetic acid," that "the excretion of mucus may precede or succeed an albuminuria," the belief in a "physiological albuminuria," also the opposing belief of recent years that this so-called physiological albuminuria was merely a nucleo-albuminuria.

Mörner used the following method of isolation:

The salts are dialyzed out of a large volume of urine and then acetic acid added, 2 cc. per litre. The precipitate is then dissolved in a little water and again precipitated with acetic acid. It may then be tested for the presence of chondroitin-sulphuric acid by heating on the water-bath with 5 per cent. HCl for a long time. If both sulphuric acid and a reducing body are present, this body is probably present. If the reducing body is demonstrated, but no sulphuric acid, it is probably mucus. If there is no sulphuric acid and no reducing body, and the precipitate then be digested with pepsin and organic phosphorus be found, the nuclein bases may be demonstrated in the products of digestion. Large amounts of urine, however, must be used for its detection.

This explanation of Mörner, satisfactory as it would seem, and evidently based on very careful work, has received little confirmation. Stähelin<sup>110</sup> in one case of jaundice failed to find any of the "albumin-precipitating bodies," and thought the precipitate resembled the globulins, a view held by Fr. Müller in 1885; also in the acetic acid precipitate of the urine of a case of pneumonia with a very heavy precipitate on adding this acid, no phosphorus could be detected. Matsumoto found it chiefly fibrinogen and euglobulin (see page 224). Oswald<sup>111</sup> studied carefully this precipitate with acetic acid in the urines of cyclic albuminurics and nephritics, and decided it to be euglobulin and a trace of fibrinogen. These occur in the blood, but cannot be demonstrated there by the addition of acetic acid, since the salt content is too low.

It is to be noted that in most of the above work small amounts of urine were used, not the large amounts of Mörner; again, that many will not agree that the limits of precipitation with saturated ammonium sulphate are alone sufficient for the recognition of a proteid. In conclusion, it may be stated that, however the present conflict between Mörner and the Hofmeister school may be settled, both agree that there is a constant normal physiological albuminuria.<sup>112</sup>

**The Nucleohiston** of Lilienfeld is a body arising from the breaking down of leucocytes. It is precipitated by acetic acid and has a high phosphorus content. This is found in the urine especially of leukæmic patients, although its appearance is not alone due to the breaking down of these cells.

Albumin, if present, is first removed; the proteids of the urine are then precipitated with alcohol, the precipitate washed in hot alcohol, then dissolved in

<sup>110</sup> Münch. med. Wochenschr., 1902, p. 1413.

<sup>111</sup> Zeitschr. f. d. gesamt. Biochem., Bd. v, 1904.

<sup>112</sup> See, also, Calvo, Zeitschr. f. klin. Med., 1904, vol. li.

boiling water, cooled, acidified with HCl, let stand, and the uric acid precipitate filtered off. To the filtrate is then added ammonia, the precipitate collected on a small filter, washed with ammonia till the wash-water gives no biuret reaction. The precipitate is then dissolved in acetic acid and tested for histon. This gives the biuret reaction, is coagulated by heat, and this coagulum is soluble in mineral acids.<sup>123</sup>

**Fibrinuria.**—Fibrinogen, fibrinoglobulin, occurs rarely in any amounts in the urine. The reactions are those of globulin, since this body belongs in that group. To recognize its presence, however, is easy, since there is a spontaneous coagulation on standing.

Excluding those cases in which there is blood in the urine, fibrinuria is rare. It occurs in chyluria and in some rare cases of nephritis. In some cases the urine clots at once after voiding, the clot being sometimes firm and in other cases gelatinous. Or this may occur before voiding, the clots being casts of the pelvis of the kidney or from the bladder (the term fibrinuria, of course, is strictly applicable only to these latter cases). In severe inflammation of the urinary passages, the bladder, ureter, or pelvis of the kidney, these clots may be formed. The reason for this is not known, since most of the inflammatory exudates do not coagulate. We have seen but one good case,—a woman admitted during the last hours of her life with what was evidently chronic parenchymatous nephritis. Only about 5 cc. of urine could be obtained. This was of a rather cloudy yellow color; no blood grossly. After standing for a few minutes it clotted to a solid coagulum. In the decomposing alkaline urine, such as occurs in alkaline catarrh of the urinary passages, masses of pus, mucus, and bacteria may be voided or may even plug the passages, and resemble fibrin casts.

FRAGMENTS OF TUMORS have also been found in the urine.

**Albuminuria.**—Cases of albuminuria may be divided into the false and the true. By the false are meant those in which the urine, as secreted by the kidney cortex is normal, and the albumin is contributed lower in the urinary passages, either as an inflammatory exudate, or lymph, blood, or chyle. By albuminuria in the following paragraphs is meant only the true,—that is, albuminuria due to some disturbance of the renal epithelium, especially of the glomeruli, not of the blood capillaries; the latter are always permeable to albumin, and the great wonder is not that albumin should ever pass through the renal epithelium, but that it does not always. Over all the rest of the body exudates and transudates are always albuminous. In albuminuria occur together serum albumin and “serum globulin.” (See page 224).

**Albuminuria without Definite Renal Lesion.**—Concerning PHYSIOLOGICAL ALBUMINURIA,—that is, the constant presence of a proteid in normal urines,—the pendulum has swung several times. Posner, in

<sup>123</sup> See Kolisch and Burion, Zeit. f. klin. Med., 1896, Bd. 29, p. 374.

1884, first claimed the presence of serum albumin in all normal urines; this was believed in and then doubted, and again accepted on the basis of certain chemical tests for albumin. With the supposed demonstration that these tests indicated rather a mucin or a nucleo-albumin, the physiological albuminuria was again doubted, until recent work, particularly that of Mörner and that stimulated by him, seems to have established beyond doubt the presence of a small amount of serum albumin, or, according to others, euglobulin, in practically all normal urines.

With Spiegler's reagent it is hard to find a person whose urine is really albumin-free. The absence of true nucleo-albumin in the urine may be considered as proof that this "albuminuria" is physiological.

If this is the case, there is no line between physiological and pathological albuminurias except that of amount. By "albuminuria" is now meant a condition in which serum albumin may be detected by the tests accepted in common use as standards, and the cases with small amounts of albumin which pass unnoticed by these tests and require special technique are not included. Hofmeister gives as standard that if Heller's test shows no ring in three minutes the urine is to be considered albumin-free.

Hence the question of albuminuria is similar to that of glycosuria, a very small amount of both bodies being normal, but disregarded unless increased to sufficient amount to give the tests accepted as criteria. The line, however, is an artificial one and very difficult to draw. This gives the teacher considerable difficulty in the medical school, in which the students are taught the very delicate tests, since each year a few discover a positive albumin test in their urine and are rendered very unhappy thereby.

Concerning this proteid of normal urine, the demonstration of which requires very delicate tests or the use of large amounts of urine, see page 225.

The above is the only correct use of the term physiological, although this is wrongly used for cases of albuminuria in the apparently healthy. By albuminuria in the following pages will be meant an amount which can be detected in the test-tube with a few cubic centimetres of urine.

But the so-called FUNCTIONAL ALBUMINURIA is a different matter. The term "functional" Pavy used merely in contrast to "structural," in which case the albuminuria depended on anatomical changes in the kidney. In these cases ordinary tests are used and a small amount of urine, and concerning the presence and nature of the proteid there is no doubt. Senator considers that the albuminuria is truly "physiological" or "functional" when it is slight in grade, occurs in young men, is transitory, the further history of that person is negative, the

urine is otherwise normal, and its occurrence follows always an unusual and adequate cause, such as very severe muscular work by those not used to it. But these cases should be placed in a separate group and the term "physiological" used with caution. Such cases are normal men who, after unusual exercise, exertion, exposure to cold, nervous stress, or after unusually large proteid meals, show a temporary albuminuria. According to Senator the cause must be something unusual for that person, and later, if he accustom himself to this cause, it will not produce an albuminuria.

This form of albuminuria was first noticed among soldiers. Leube stated that 59 per cent. of raw recruits showed a temporary albuminuria after a forced march, which later failed to appear after such a march.

Macfarland<sup>114</sup> found in practically every foot-ball player after a game an albuminuria which lasted for the most part but three to four hours. Müller<sup>115</sup> showed that eight of eleven bicycle riders after races showed albumin, and seven of twelve showed casts of all descriptions and renal epithelium. The urine was normal the following day. Barach\* found in the urine of every one of nineteen Marathon runners, albumin, casts, and, in nearly all cases, blood. One week later the urine of four of these men still contained casts and albumin, and of two casts alone. The same is true of athletes, mountain climbers, bicycle riders, foot-ball players, those persons who exercise severely the leg and thigh muscles especially to a degree beyond that to which they are accustomed, and later are able to stand an equal amount without the same result. We may say that it is only a question of limit; practically every one can, if he will, produce albuminuria, if he only over-exerts himself sufficiently. The most normal man in every sphere of life must still observe certain limitations, and the question comes, Having overstepped these, can the albuminuria which results be termed "physiological"? Of course, the limits for persons differ, and what is physiological for one is pathological for another, but the groups of cases now under consideration concern only those of the highest physical attainments,—trained athletes, young men picked for the army, etc. Macfarland's studies of the urines of twenty foot-ball players immediately after a game showed that for a while at least after severe exercise the kidneys are not in normal condition. In the urine of nineteen he found granular casts; in that of six, blood casts and red blood cells.

In this same group Senator includes cases the relation of which to the normal bounds of the physiological it is more difficult to deter-

<sup>114</sup> New York Med. Rec., 1894, vol. xlv, p. 769.

<sup>115</sup> Münch. med. Wochenschr., 1896, No. 48.

\* Arch. Int. Med., April, 1910, vol. v, p. 382.



mine. Among these are the albuminurias which follow violent emotions and an unusually heavy proteid meal. The latter, the true "alimentary albuminuria," is a form doubted by many who do not believe that it is a normal function of the kidney to excrete an excess of protein just as it relieves the blood of an excess of glucose. Among soldiers, hence men under uniform conditions, Rapp found that 10.7 per cent. showed albuminuria after their mid-day meal.

Experiments show that the ingestions of large amounts of certain proteids will in some apparently normal persons cause albuminuria; but the amount of most proteins ingested must be excessive to have this result. Much smaller amounts of egg albumin than of other proteins can be detected in the blood-plasma, and in nephritis small amounts are excreted through the kidneys. The output of albumin in normal men who have overeaten begins in about two hours and lasts four.<sup>116</sup> Animal experiments, however, have shown that when egg albumin is excreted serum albumin is as well. This has been proven both chemically and by the specific precipitines (it is only just to say that this method [precipitines] has not proved very satisfactory). The conclusion is that the excess of proteid in the blood may have, temporarily at least, injured the kidneys. An alimentary albuminuria is claimed for the new-born fed on cow's milk, whose intestinal mucosa has not yet developed that impermeability to foreign proteids which later is present.

Prolonged cold baths will cause albuminuria. Rem Picci<sup>117</sup> found from observations on one hundred and fifteen baths of thirty-five healthy men that three minutes at 12° to 13° C., or fifteen minutes at 15° to 20° C. (none at 20°), caused quite regularly a slight transitory albuminuria, minimal in amount, never lasting over twenty-four hours, with casts, and generally diuresis with increased urea and chloride output. This he explained from reflex nervous influences from the skin. It is serum albumin in the urine.

Mental over-exertion is also claimed as a cause in certain cases.

There is special reason for the albumin to appear should several of these predisposing factors occur simultaneously. The intermittent nature of the albuminuria is no criterion, since a truly pathological case may intermit considerably; but in all such cases must be emphasized the appearance of albumin after a very unusual strain or occurrence, and one adequate to explain its appearance; also its very temporary duration. Senator considers that if the amount of albumin exceeds 0.4 to 0.5 gm. per litre it cannot be called "physiological."

<sup>116</sup> For recent articles, see Ascoli, *Münch. med. Wochenschr.*, 1902, No. 10, and Inouye, *Deutsches Arch. f. klin. Med.*, 1902, Bd. 75; and on the opposite side of the question Umber, *Berl. klin. Wochenschr.*, July 14, 1902; for the chemical side, Sollman and Brown, *Jour. of Exp. Med.*, March 17, 1902.

<sup>117</sup> *S. J.*, 273, p. 37, 1901.

Another example of "physiological" albumin is the ALBUMINURIA OF THE NEW-BORN. Often for the first eight or ten days there is a slight amount of albumin with hyaline casts, epithelial cells, and urates present. This is also present in the urine found in the bladder of still-born children, and therefore is not attributable to any changed circulatory or metabolic products after birth. Ribbert gave as an explanation that the kidneys at birth are really not quite "finished," but there still occurs a desquamation of epithelium of the capsules of the glomeruli, hence with the albumin occurs nucleo-albumin from these cells.

The ALBUMINURIA OF WOMEN IN LABOR should be considered as physiological. Some find that in about 39 per cent. of normal cases this is present. It is attributed to the circulatory changes of the kidney due to the work, strain, etc. The condition of the kidney is doubtless pathological, but the cause is physiological, and the albumin usually disappears at once. Little,<sup>118</sup> as the result of very careful work, concludes that albumin is present in the catheterized specimens of urine from about one-half of all pregnant women, being equally frequent in primiparæ and multiparæ. Casts occur with greater relative frequency in multiparæ. During labor these percentages increase, especially in primiparæ. This may be due to the muscular work and increase of blood-pressure during labor. During the puerperium the percentage drops.

"ALBUMINURIA OF ADOLESCENCE" (Gull), "of puberty," "accidental albuminuria," "essential albuminuria," "physiological albuminuria," "Pavy's disease," "cyclic albuminuria of the apparently healthy," "postural," "orthotic," "orthostatic," and "intermittent albuminuria." This group of cases is of far greater importance and interest than the preceding. It is also a much larger group than has been suspected. These cases are discovered by army medical inspectors, by the examiners for insurance companies, and by the doctors to whom our neurasthenics apply for treatment. Insurance men say that of the "normal" persons examined while the temperature is above 90° or below 0° F., 5 per cent. show albumin; at other times, about 2 per cent.

This group includes those persons enjoying reasonably good health, but whose urine either constantly or temporarily contains a trace of albumin. Their number is large, and they certainly can be divided into several groups, which classification it is convenient to use, although the present may strike entirely beyond the bounds of evidence. The above long list of names shows what features may predominate. Posner proposes the quite satisfactory term, "essential albuminuria," for the albuminuria is the one symptom common to all.

<sup>118</sup> Amer. Jour. of Obstet., vol. 1, No. 3, 1904.

Of the group as a whole it may be said that it includes young persons during adolescence or in the few following years, who are often not of best health, and not robust but anæmic, often children with a neurotic family history and with unstable vasomotor system, who sometimes give in their history such diseases as scarlet fever, diphtheria, et al., which would suggest a latent nephritis, who may continue for years in good health or later show clear signs of Bright's disease. Common to all is the absence of other signs and symptoms of kidney trouble, and if it is cyclic or intermittent the albumin appears in response to ordinary acts of our every-day life, that is, not to an unusual or adequate cause. In some cases it is said to follow walking or other exercise, in others a heavy meal. It is sometimes a family disease, three children in one family showing it (Lacour). In this group are included by some the albuminuria of masturbators and that following sexual excitement. In these cases it may be present only before rising in the morning. Some (Sir Andrew Clark and others) say this proteid is a secretion of the ureter or accessory glands.

A diagnosis is possible only after long careful study of the individual case, including past history and especially the physical signs on the part of the heart and eyes, and even then the autopsy may reverse the diagnosis.

If there be good evidence of past renal disease or any cardiac features suggesting it, the case must be considered one of nephritis. The specific gravity, amount, sediment, etc., of the urine are important in diagnosis. The intermittent nature of the output is no criterion, since this may be seen in true chronic nephritis; nor does the presence or absence of casts help, since hyaline casts may occur whenever albumin does, and careful search shows them in the more truly physiological cases; nor does the typical postural character, for this is seen in cases of acute nephritis after scarlet fever as it recedes (Knöpfelmacher) and in cases of chronic interstitial nephritis and of waxy kidney.

While one case may fall in any one or several of the following groups, we give the classification to emphasize the features which such cases present.

The "ALBUMINURIA OF ADOLESCENCE" is a form separated by Leube from the one great group. It occurs between the ages of fourteen and eighteen years and then disappears. It may be explained by a renal insufficiency relative to the growing organism, the kidney not keeping pace with the physical growth and activity, together with instability of the vasomotor centres. In this group occur most of the cyclic or postural cases, not all, since some of these latter continue to adult life, and not all the cases of this group are truly

postural. The element of heredity is very important. The cases reported by Lommel<sup>119</sup> would fall under this title, since the question of posture was little considered. Of 587 factory workers from fourteen to eighteen years old, 18.9 per cent. showed albuminuria once or many times, in small amounts and for the most part intermittent. Of sediment there was none, or at the most a few hyaline casts and fatty epithelial cells in the centrifugalized specimen. Of 130 patients from the same class, but over twenty-five years old, only one showed albuminuria. Cardiac and vascular disturbances were common. Posner emphasized sexual excesses at puberty as a common cause. Sutherland<sup>120</sup> emphasized the relation between this form and movable kidney present in one-third of his cases, and so common in children.

CYCLIC ALBUMINURIA is the most interesting of all. This form shows a remarkable daily cycle, the albumin being absent at night and when the patient is flat on his back, but appearing when he stands up. The terms ORTHOSTATIC or POSTURAL are therefore more suitable. It is the history, extending over considerable time, and the negative physical examination which permit us to place these cases among the functional albuminurias. Other cases of albuminuria may be beautifully cyclic, yet definitely pathological. Such is seen in cases of beginning nephritis, and during the convalescence it is, in fact, a very suggestive sign of Bright's disease.

The rest of the cyclic cases may be subdivided: into those associated with vasomotor phenomena and with the neuropathic element predominating; those with circulatory derangement, as congenital floating kidney; and the hereditary form (Mix). As a rule, the albumin appears after rising, and reaches a maximum at noon or from 4 to 6 P.M., then declines, disappearing from 8 to 10 P.M. If the patient change his habits, the cycle will change as well. Many cases bear little or no relation to meals. There is not only a cycle of the albuminuria, but also diminution in water output, and the sequence each time is, increase in pigments, of albumin, of uric acid, lastly, of urea (Teissier). While casts are rare, yet careful search will, as a rule, show them. The albuminuria may even be diminished by exercise and fatigue, hence is less at night after a hard day's work. Mix<sup>121</sup> has divided such cases into the intermittent and continuous, the terms applying to the periods over which the daily cycle occurs. In the continuous form the cycle continues for years, and if it ceases does not recur. These cases practically never develop in Bright's disease. The adults are neurasthenics with vasomotor paresis, and

<sup>119</sup> Deutsches Arch. f. klin. Med., 1903, Bd 78, p. 541.

<sup>120</sup> Am. Jour. of the Med. Sci., 1903, vol. cxxvi.

<sup>121</sup> Ibid., 1904, vol. cxxviii, p. 307.

the children in about 37.5 per cent. of the cases have a congenital movable kidney.

There has been a great dispute whether such cases should be considered as pathological or not. They are rare. Other transitory albuminurias are certainly pathological; *e.g.*, those of fevers, and even in these cases is there not always a subsequent history. Most writers warn against considering them as functional, since the duty of renal epithelium is to retain albumin, and when it does not do so something is wrong. Senator says the patients are chiefly young people at puberty of not the best health; they are anæmic, weak, and faint easily. Armstrong,<sup>122</sup> from the study of over three thousand school-boys, found this form in 12 to 15 per cent. It is found more in summer than in winter; heredity is often present; it is often associated with depression of spirits and fainting spells, especially while the boy is standing idle, not when occupied; the boy is apathetic, with a heart subject to intermittent attacks of dilatation and palpitation; it lasts only during puberty. Posner's case was well after seventeen years. Such may, perhaps, be considered as persons renally weak, hence like certain cases of glycosuria, and yet who give no subsequent history. Senator<sup>123</sup> still insists that the majority of these cases are nephritis either at onset or during a latent case. Krehl, having followed several cases over a long period of time, considers that the absence of subsequent history of nephritis allows us to consider the condition harmless; that these are not mild cases of Bright's disease. Broadbent<sup>124</sup> has never known a true case of this form to develop actual renal disease. In all the above cases the amount of albumin is small, the amount of urine normal, with the specific gravity normal; a few hyaline casts are sometimes present; in others no casts are found at any time after careful search; and there are no cardiovascular changes. The immediate cause is much in dispute. Possibly the most reasonable explanation will be one which associates the urinary findings with the rather marked changes in the renal circulation which follow changes in posture. Edel in three very interesting cases found that the albumin-free intervals (in the afternoon as a rule) were also periods of diuresis. He found that the amount of albumin varied roughly inversely to the amount of urine, and thought that diuresis was the chief thing to strive for. He emphasized the relation between the condition of the urine and that of the heart, the albumin being absent when the pulse was "full." This question is best studied by Erlanger and Hooker,<sup>125</sup> who found that the

<sup>122</sup> Brit. Med. Jour., 1904.

<sup>123</sup> Deut. Arch. f. klin. Med., December 8, 1904.

<sup>124</sup> Brit. Med. Jour., 1904.

<sup>125</sup> Johns Hopkins Hosp. Rep., vol. xii, 1904.

amount of albumin varied inversely as the pulse-pressure (the difference between the systolic and diastolic pressures), the albumin appearing when this is low. Many of these cases later recover. The last and best review of this subject is that of Hooker.\*

Many cases which first are included in this group later develop the typical signs of Bright's disease.

The HYPOSTATIC ALBUMINURIA of splenic origin which occurs in some persons with enlarged spleens while recumbent, and is absent while erect, is, Rolleston thinks, the opposite of cyclic albuminuria. Since it is not seen in all with enlarged spleens nor in those with the largest, some other factor is necessary. The pressure on the left renal vein may explain it. It may resemble the albuminuria in the chronic passive congestion of mitral disease.

ALBUMINURIA MINIMA (Lecorché and Talamon).—Under this group are included cases with a constant trace of albumin, almost never 0.5 gm. per litre. The output is quite constant in amount, varying little with the position of the patient, the time of day, diet, etc. For each case, however, there may be some factor which causes an increased output. Some such cases are quite certainly the result of a preceding acute nephritis, a residuum as it were. Their prognosis is uncertain and must be guarded, for some develop to true nephritis. Others remain the same for years with no further symptoms.

Under this group the French put the post-infectious cases, albuminurie résiduale, albuminurie paracellaires (or insular nephritis), albuminurie cicatricielle (due to imperfect healing, leaving a "scar"); also the albuminuria of adolescence, the hereditary form, albuminurie phosphaturique, and the albuminurie prégoutteuse.

INTERMITTENT ALBUMINURIAS are those in which periods with albumin are followed by others with clear urine. This term does not include the cyclic or postural, which terms are limited to those with daily periodicity, while the periods of the intermittent may extend over weeks and months or years. The term "intermittent" is more applied to cases of temporary albuminuria due to a known cause. These cases are usually of insidious nephritis, and give a history of some acute infectious disease. But one of the best illustrations is the albuminuria accompanying heart disease. These patients are admitted repeatedly with broken compensation and urine containing albumin and casts which soon disappear.

The *intermittent hereditary form* includes, according to some, many cases of the albuminuria of adolescence, the cases showing none in adult life except in response to fairly adequate cause.<sup>126</sup> In some

\* Arch. Inter. Med., May, 1910, vol. v, p. 491.

<sup>126</sup> Dieulafoy, Loude, Arch. gén. de méd., n. s., ii, 3, p. 257, 1899.

cases the parents had albuminuria during youth, while in others a neurotic family history is the only suggestive feature.

**Traumatic Albuminuria.**—Transitory albuminuria follows injury to the brain, apoplexy *e.g.*; after injuries crushing the kidneys the albuminuria and casts may continue for a long time with no other signs of nephritis. This may explain some cases of benign latent contracted kidneys (Stern, Curschmann). Menge<sup>127</sup> found that even bimanual palpation of the kidney in physical examination will in 15 of 21 cases cause a transitory albuminuria lasting usually from one to twenty-four hours at the most, and in some cases a slight hæmaturia. Anything obstructing renal venous flow, as in movable kidney during the crises, may cause albuminuria and cylindruria.

**Febrile Albuminuria.**—During any acute fever, but especially pneumonia, typhoid, malaria, acute articular rheumatism, grippe, or even tonsillitis, there may be a slight albuminuria, simultaneous with the rise in temperature and disappearing with its drop. In such cases the cloudy renal epithelium, the faintest grade of inflammation (Leyden), is considered the anatomical basis. The amount is usually small, but sometimes great. Hyaline and epithelial casts are sometimes found, but no other formed elements indicating inflammation. In general it is only a matter of degree which separates these from true cases of nephritis.

Under hæmatogenous albuminuria is included a very confusing group of non-febrile cases which show at autopsy no renal lesions, except, perhaps, slight parenchymatous changes. In this group are cases of purpura, scurvy, chronic lead or mercury poisoning, lues, leukæmia, cachexia, and anæmia in which the albuminuria is always slight and occurs only when these conditions are severe, cholæmia, glycosæmia, and following ether and chloroform narcosis.

Strictly speaking, "hæmatogenous albuminuria" should mean one in which, either due to some alteration of a normal proteid of the blood, or because it is foreign, a proteid unsuitable for use is excreted. All cases with the possibility of the presence of a toxic influence on the kidneys, for instance, lead, mercury poisoning, etc., should be excluded, since such would have a truly renal origin. It is true that foreign proteids in the serum are excreted, *e.g.*, albumoses, egg albumin, peptone, casein, free hæmoglobin, etc. Some consider that in all cases of albuminuria such is the case, an abnormal proteid or a normal one rendered unfit for further use being merely excreted. Yet in cases of true nephritis there is no evidence of a foreign proteid or qualitative change of the normal proteid. It is suggested, however, that quantitative changes either of proteids or salts could explain the albuminuria. But more probably the real cause is in the cells them-

<sup>127</sup> Münch. med. Wochenschr., June 5, 1900.



selves, the renal epithelium being exceedingly sensitive to changes in its nutrition, and that a true hæmatogenous albuminuria is not proved.

**The Nervous Form.**—Epilepsy, apoplexy, tetanus, exophthalmic goitre, injuries to the head, delirium tremens, various psychoses, even neurasthenia and migraine may be accompanied by a slight transitory albuminuria. In some cases there are a few casts present. Interestingly enough in other cases only casts are to be found. We followed the urine of such a case in a boy fourteen years old with hysterical attacks. A very transitory albuminuria cannot be excluded, since the urine may not have been examined early enough. The cylindruria lasted for several weeks.

Cases of closure of the ureter, retention of urine in the bladder, compression of the thorax, have been accompanied by albuminuria; digestive disturbances, as obstruction of the bowel (a reflex cause being assumed as in cases of strangulation of bowel or omentum;<sup>128</sup>) acute diarrhoea, constipation, and liver disease are sometimes given as causes. In two-thirds of the cases the albumin disappears after the obstruction is relieved even though the bowel has been rendered gangrenous. The cause is uncertain. It is probably not the absorption of any bodies, since in peritonitis, where there would be a similar absorption, the albuminuria does not occur. Such cases are transitory.

**Albuminuria with Definite Renal Lesions.**—In active renal congestion, as after exposure to cold, or in chronic passive congestion due to heart or lung disease, tumors, or pregnancy, albumin may be present, yet no other renal lesion found. As a rule the albumin is little in amount and this runs parallel to the amount of urine, while in the case of true nephritis the amount varies inversely as the amount of urine as a rule. In children albuminuria may accompany the simple hyperæmia in diphtheria, *e.g.*, which may then stop or develop into a nephritis.

**ORGANIC BRIGHT'S DISEASE** of all varieties is accompanied by albuminuria at some time during its course. It is interesting that there is no parallelism between the amounts of albumin and the severity of the nephritis. In the chronic interstitial nephritis ending in uræmia it may be present in traces. In other cases periods with traces may alternate with months when there are none. In general the rule is that the more acute the case the larger the percentage of albumin. In all cases it is, however, more a matter of percentage than of total albumin; for to excrete a larger amount of urine with a lower percentage of albumin is evidence of a better renal condition than previously when the percentage was higher but the total output much smaller since the output of urine was diminished. In some cases definitely acute there may be no albuminuria.<sup>129</sup> In nephritis also the

<sup>128</sup> Neumann, Trans. Clin. Soc. of Lond., 1897, Bd. 30, p. 65.

<sup>129</sup> Herringham, Trans. Clin. Soc. of London, vol. xxxiv. p. 901.



percentage of albumin varies, as a rule, inversely to the amount of urine. The albumin is seldom present in amounts of more than 1 per cent. Sometimes it reaches 2 per cent., while in very rare cases 5 per cent. or, in one case, 8 per cent. Senator mentions a case of subacute nephritis with a percentage of from 6 to 8 per cent. over a period of some days. Cases with the largest amounts of albumin output are interestingly enough often due to lues. These cases of nephritis syphilitica acuta præcox are rare, but between 20 and 25 are recorded. In Hoffmann's case the enormous albuminuria ran parallel to the luetic symptom and improved under mercurial treatment.

Salkowski's case <sup>180</sup> is especially interesting. The urine had a specific gravity of 1056, and 7 per cent. proteid. On standing, there was deposited a rich white amorphous precipitate, not a coagulum, of a proteid giving reactions between globulin and an albuminate, and which after standing gave those of albumin. This same case on another day showed even 8.5 per cent. albumin (the blood contains but about 7.5 per cent. of proteid).

The total output of albumin is seldom great, that is, more than from 1 to 20 gms. The deleterious effects of the nephritis cannot be attributed to the actual loss of albumin, since this loss, as a rule, can be easily covered by one good meal. In amyloid disease the amount of albumin may be great or very small; as a rule from 0.5 to 0.05 per cent.

This albuminuria varies much, there being definite waves in the output. At first indiscretion in diet increases it, probably by intensifying the acute element of the process, later a more liberal diet may improve the condition. In some cases it would seem as if meat were not as harmful as vegetables, perhaps due to the salts of the latter. The albumin is increased by the erect posture, but this does not explain its increase during the waking hours, since the same curve is presented by patients who are semierect all the time as by those who can rest recumbent. Exercise of any kind, even massage (Edgren), increases the output.

A pure milk diet sufficient to cover the heat-needs is injurious, causing even hæmaturia, and should be varied with other nitrogenous foods.

**"Hetero-albumosuria." Bence-Jones' Body. "Kahler's Disease." "Myelopathic Albuminuria of Bradshaw."**—This body, which occurs in certain rare conditions in the urine in very large amounts, was supposed, from some of its chemical properties, to belong to the hetero-albumoses. Some recent work, for instance that of Magnus-Levy who obtained it crystalline, showed that it is nearly related to genuine albumin. Among other reasons for this is that its digestion products include all the primary proteoses except hetero-

<sup>180</sup> Berl. klin. Wochenschr., March 3, 1902.

albumose. This throws considerable doubt on the belief that it is a primary digestive product itself. Lindemann concludes that while it cannot without objections be put in any group of proteids it is nearest the true albumins. Dechaume considers it a mixture of at least three proteids (or groups of proteids),—proto-albumose, dysalbumose, and a body like hetero-albumose. In 1903 but about 35 well studied cases had been reported. All but one (Askanasy), and this a case of lymphatic leukæmia, were cases of multiple myelomata. In all cases there is extensive disease of the marrow. Such cases run a rather acute course with a fatal termination in from one-fourth to one and a half years.

The Bence-Jones' body is often present in large amounts, even 7 per cent., but in the majority of cases it is below 1 per cent. Some cases are reported as intermittent (Boston). Coriat<sup>181</sup> reported a case with none in the urine, but with 4 per cent. in the pleural fluid.

REACTIONS.—The specific reaction is that on warming there develops at a low temperature (about 60° C., often 52°) a milky, then heavy, sticky precipitate, which disappears for the most part and in some cases perfectly on bringing to a boil, and reappears on cooling. The urine must first be rendered acid with acetic acid. This being the characteristic reaction, the name suggested by Hugounenq for the condition "thermolytic albuminuria" ("albuminurie thermolytique") is very appropriate.

On adding nitric acid to the urine a heavy precipitate forms which is soluble on warming and reappears on cooling.

The urine may be saturated with  $(\text{NH}_4)_2\text{SO}_4$  at 100° C., filtered: the precipitate washed with saturated  $(\text{NH}_4)_2\text{SO}_4$ . The precipitate is then dissolved in water or dilute NaCl solution and the biuret test applied. The urine gives the biuret reaction directly.

These are most striking reactions and attract attention at once. The precipitate appears at a moderately low temperature, which depends on the amount present, also on the salt content of the urine. While a definite temperature cannot be stated, it is in general below 60° C. In the different cases the properties of the substance found have differed so much that either they were different bodies, or were not tested pure, or, and this is the present view, the varying amounts of salts and urea affected the tests. The urine may be turbid when voided.

Boston<sup>182</sup> proposed the following test based on the large amount of loosely bound sulphur it contains. From 15 to 20 cc. of urine in a test-tube are mixed with an equal amount of saturated NaCl and shaken to a perfect mixture. Then 2 to 3 cc. of 30 per cent. NaOH are added and the tube shaken hard. The urine at the top of the tube

<sup>181</sup> Am. Jour. Med. Sci., 1903, vol. cxxvi.

<sup>182</sup> Ibid., 1902, vol. cxxiv.

is then heated to boiling and PbAc solution (10 per cent.) added drop by drop, heating after each drop. In one-half to one minute one gets a brown color turning to black.

The output of this body is quite constant during the day and is not affected by diet, hence it is probably not a non-assimilated product of digestion. It seems to be formed in the bone-marrow. Some connection with the granules of the myelocytes and tumor cells is suggested.

This albumose can be demonstrated in ascitic fluid, blood, and bone-marrow.

Quantitatively the Esbach tube will give an approximate determination.

For recent literature concerning the nature of the substance the reader is referred to Simon.<sup>133</sup>

**Albumosuria, "Peptonuria."**—Under this term at least two different groups of bodies have been described,—the above-mentioned rare so-called "Bence-Jones' body," which because of its many reactions was counted with the primary proteoses, and a group of bodies having nothing in common with the above, formerly called peptones, a name based on Brücke's definition as a proteid not precipitated by  $K_4FeCN_6$  and acetic acid. By "peptone" is now generally understood one not precipitated by complete saturation with  $(NH_4)_2SO_4$  (Kühne), and judged by this standard these bodies are chiefly deuterio-albumoses, hence the name "peptonuria" is less used and "albumosuria" has taken its place. But this criterion is not satisfactory (Neumeister). As judged by it true peptone has been demonstrated in the albumosuria of croupous pneumonia, ulcer of the stomach, pulmonary tuberculosis and during the puerperium. It occurs always with albumose (the reverse is not true).<sup>134</sup>

In testing for the deuterio-albumoses the urine should be albumin-free, and if this is not the case it should be made so by the Hofmeister method. Mörner's body may be precipitated by basic lead acetate.

An easy test is to saturate the urine with ammonium sulphate, a flocculent precipitate indicating albumose.

A good preliminary test for the deuterio-albumoses is that of Hofmeister. To the urine is added one-fifth volume of concentrated acetic acid and then phosphotungstic acid. If the urine remains clear after standing for some time, these bodies are not present, while a milky cloud at once or in about ten minutes indicates them. This test is valuable if positive, but not if negative.

The biuret test is that usually used. According to Hofmeister the albumose is first precipitated with tannic acid or phosphotungstic

<sup>133</sup> Am. Jour. Med. Sci., 1902, vol. cxxiii, p. 939.

<sup>134</sup> Ito, Deutsches Arch. f. klin. Med., 1901, vol. lxxi.

acid. The precipitate is dissolved in a little water and the concentrated solution tested. NaOH or KOH are added in excess and then very dilute  $\text{CuSO}_4$ . Deutero-albumoses are indicated by a violet-red color. It may be necessary to filter off the precipitate of  $\text{Cu}(\text{OH})_2$ . If this test be applied to the urine directly, the color obtained is a red or a reddish-brown, the violet being obscured by the color of the urine. The test may also be performed as a contact test, the urine being rendered alkaline and then carefully superimposed by a dilute  $\text{CuSO}_4$  (5 cc. of a saturated solution to 1 litre of water).

For a positive test the albumose must be isolated. This may be done with phosphotungstic acid, which will allow 0.1 gm. per litre to be detected, or tannic acid, somewhat less delicate. Albumin and "nucleo-albumin" must first be removed with basic lead acetate.

Salkowski's method. This was designed to detect small amounts of albumoses. To 50 cc. of urine in a beaker is added 5 cc. of concentrated HCl or acetic acid. It is then precipitated with phosphotungstic acid and warmed over the free flame; the precipitate collects as a tough resinous mass at the base of the beaker. The supernatant fluid is decanted and the precipitate washed with distilled water a few times (twice), being careful that none be lost. On the precipitate is then poured 8 cc. of water plus 0.5 cc. of NaOH (sp. gr. 1.16). It dissolves readily. The blue solution is then warmed until clear. More NaOH is added if necessary, since the mixture is often of a dirty grayish-yellow color and cloudy. The solution is then cooled and the biuret test applied by adding in a test-tube a few drops of 2 per cent.  $\text{CuSO}_4$ . Before applying the biuret test Sahli recommends that the colored fluid be cleared with  $\text{BaCl}_2$ . Urobilin, if found with the spectroscope, must be removed, and may be extracted with amyl alcohol. Sahli says that it is completely enough precipitated with  $\text{CaCl}_2$ . The test may be performed in five minutes. The small amount of urine used minimizes the danger of mistake with "nucleo-albumin."

Another method is as follows: To the urine is added 0.1 volume of concentrated HCl, then PWO acid, again HCl, and again PWO acid, until neither gives any more precipitate. The urine is then filtered at once before uric acid precipitates. The precipitate is washed on the filter with  $\text{H}_2\text{SO}_4$  (3 to 5 volumes concentrated  $\text{H}_2\text{SO}_4$  in 100 cc. of water) until the wash-water runs colorless. The moist precipitate is then rubbed up with dry  $\text{Ba}(\text{OH})_2$  in excess. A little water is then added and the warm solution filtered. If heated too much the solution becomes dark. The peptone solution is always yellow. The biuret test is then applied and a red color obtained if albumose be present. In this case the test is best performed as a contact test, since the  $\text{BaSO}_4$  precipitates and settles.

Hammarsten recommends the following method, which has been modified by Bang: Ten parts of urine plus 8 parts of saturated ammonium sulphate are heated to boiling for a few seconds. The hot fluid is then centrifugalized from one-half to one minute and decanted. From the precipitate is extracted the urobilin with alcohol. The residue is then taken up with little water, heated to boiling and filtered. This removes the albumin. It is then shaken out with chloroform to remove the last trace of urobilin. The chloroform is then pipetted off and the water tested with the biuret for the presence of albumose. This is a very practical clinical method.

Alder,<sup>135</sup> after criticising all the preceding, recommends the following as more accurate. Albumin if present is removed by trichloroacetic acid (15 per cent.). To 6 to 10 cc. of urine in a test-tube are added 1 to 2 drops of HCl till acid, then 5 per cent. phosphotungstic acid till complete precipitation. The fluid is then centrifugalized for a few seconds. The supernatant fluid is poured off, the sedi-

<sup>135</sup> Berl. klin. Wochenschr., 1899, pp. 764, 780.

ment suspended in absolute alcohol, and again centrifugalized. This is repeated till the sediment and the alcohol (colored yellow with urobilin) are white and clear. The sediment is then suspended in water, strong NaOH added, the fluid shaken till all blue color disappears, then the  $\text{CuSO}_4$  is added. 0.2 gm. per litre can be detected.

**OCCURRENCE.**—The deutero-albumoses may occur either alone or with albumin. In cases of nephritis the albumose is said to accompany the albumin. It may however precede the albumin or continue after it has disappeared. The reason for this is not known. Since the urine contains a pepsin-like ferment the formation of albumose by the digestion of albumin may be suspected.

*Hæmatogenous Albumosuria.*—Many think that this group includes nearly all cases. When there is considerable albumose in the blood some is excreted in the urine, but not when the amount in the blood is small. The source of this albumose is thought to be disintegrating cells. These may be blood cells, as in leukæmia, scurvy, purpura, during the absorption of hemorrhage, during the action of a hæmolytic poison, etc. Or they may be tissue cells destroyed by disease or by some toxine. The occurrence of albumosuria during pregnancy is interesting. That during the puerperium is ascribed to the involution of the uterus, and that after the death of the foetus to the maceration of the infant, but it occurs also in some cases of normal pregnancy.

Enterogenous albumosuria is seen in cases of gastric or intestinal ulcer, as, *e.g.*, in intestinal tuberculosis. In such cases small amounts of albumose ingested, *e.g.*, somatose, will give a positive test; normally larger amounts are necessary (alimentary albumosuria). Some consider that if following the ingestion of from 40 to 60 gms. of albumose this body be found in the urine it is in favor of a gastric or intestinal ulcer.

In nephritis, especially luetic, albumosuria occurs. The "hepatogenous" form occurs in acute yellow atrophy, cirrhosis, cancer, catarrhal jaundice, and phosphorus poisoning. The "febrile" form occurs in fevers, especially the infectious; rheumatism, septicæmia, typhoid, phthisis, gangrenous processes, measles, scarlet fever, erysipelas, and smallpox, especially as the temperature falls. It occurs in mental diseases and paralyses. "Pyogenic albumosuria" is supposed to be due to the absorption of an exudate, as in pneumonia during resolution, in empyema, bronchiectasis, epidemic cerebrospinal meningitis, abscess, and osteomyelitis. Gangrenous processes anywhere may cause it, also cancers of any organ.

The common element in most of these conditions is the breaking down of some tissue or exudate, *i.e.*, increased catabolism such as occurs in all fevers and in cancers (Aldor) or exudates.

There are certain sources of albumose which should always be excluded; as, for instance, spermin and secretions of the accessory

genital glands; the foods, since on a milk diet in nephritis it is claimed that the products of digestion are absorbed and excreted unchanged; and lastly, that due to technique in removing albumin from the urine. Clinically, the deuterio-albumoses are important only when the urine is albumin-free. In albuminuria it may nearly always be demonstrated and the question arises whether it was preformed or formed from the albumin by the technique. The amount formed in this way, however, if the work be done well should be very small.

It has very little clinical value, since it has such a wide occurrence. It could be of value, however, in a case of suspected abscess (*e.g.*, of the appendix, brain, or an empyema); or in the differential diagnosis of tuberculosis and epidemic cerebrospinal meningitis. The amount is always small when compared with that of the Bence-Jones' body.

**Hæmaturia.**—This may be a symptom of the following conditions:

(1) General diseases: the malignant forms of acute specific fevers, especially smallpox, typhoid fever and malaria; in leukæmia occasionally; in the so-called hemorrhagic diathesis, hæmophilia, scurvy, morbus maculosus Werlhofii, and the purpuras. In the latter diseases the process may be limited to the kidney.

(2) Renal causes: acute and chronic congestions and inflammations of the kidney; all forms of nephritis at the onset, especially one called the "hemorrhagic" form. Nephritis due to turpentine, carbolic acid, and cantharides especially has a hemorrhagic onset. In purulent nephritis traces only of blood may be present. The chronic parenchymatous nephritis Weigert considered always hemorrhagic, with small amounts of blood constantly present in the urine. In amyloid disease there are few or no red blood-cells in the urine. In chronic passive congestion due to various causes there may be blood in the urine. In renal infarctions there may be considerable bleeding, but that is rare; in new growths of the kidney the hæmaturia sometimes is profuse; at the onset of renal tuberculosis, especially if the papillæ are involved; in cystic kidneys, renal calculus, and lastly, in parasitic diseases of the kidney, especially filaria, echinococcus and the distoma hæmatobium. In congestion due to venous thrombosis, *e.g.*, of the new-born, hæmaturia is said to be common.

(3) It is also found as the result of lesions or diseases of the urinary passages, as for instance, stone in the ureter, tumors and ulcers of the bladder, parasites of the bladder, calculi and ruptured veins; in urethritis.

(4) In trauma of any part of the urinary tract from the kidney down.

(5) And lastly an interesting group with no known lesion. The so-called "Gull's renal epistaxis" or "essential renal hæmaturia," or

“angioneurotic hæmaturia,” or “renal hæmophilic,” is a rare disease of middle adult life, often unilateral. In certain of these cases angiomata of the kidney have been found, in others none, and nervous causes are suspected. Some of these cases recover without further treatment; others after treatment of the nervous system, while others after a nephrotomy, or nephropexy or simple exposure of the kidney.<sup>136</sup>

In women the vagina as a source must always be excluded.

More recent work with microscopic examination throws some doubt on the normal nature of these kidneys. Eshner<sup>137</sup> collected 48 cases of unilateral renal hæmaturia, most of which had been diagnosed as calculus or cancer. Since then other interesting cases have been reported. A diagnosis of unilateral hemorrhagic nephritis was made in Stich's case.<sup>138</sup> In Schüller's case the kidney looked normal, but microscopically chronic parenchymatous nephritis was found.<sup>139</sup>

The term “hæmaturia” is used only when blood is grossly visible. The urine is always turbid, of a light smoky to a bright red or blackish-brown color. Microscopically are found the red blood-cells in various conditions of preservation, and other elements according to the cause. In renal hæmaturia clots are seldom present, the urine and blood are homogeneously mixed hence in equal amounts in the two-glass test, while in cases of hemorrhage from the bladder the second glass will contain the more blood, and if the bladder be washed out the washings will be blood-stained, while in renal cases, clear. In acute exacerbations of a chronic parenchymatous nephritis especially, the amount of blood in the urine may be considerable. Clots are present in rare cases, as when large vessels of the kidney rupture, or in cases of aneurisms, trauma, or varices. In a case in the ward recently a clot four inches long, a cast of the ureter, was voided. Such clots are more common in cancer than in calculus.

Gerhardt thinks that the blood-cells from the kidney are more spherical, more leathery in color than usual, while all the morphological elements from the kidney, the casts and epithelial cells, are yellowish-brown. In renal hæmaturia are found also casts of various kinds, blood-casts, or casts with red cells attached, and renal epithelium, showing parenchymatous lesions. Albumin will also be present. It is generally believed that if the blood be not from the cortex and the urine allowed to settle, the clear supernatant fluid will be albumin-free.

**Hæmoglobinuria** is the result of hæmoglobinæmia, or the destruc-

<sup>136</sup> Stavelly, Johns Hopkins Hosp. Bull., March, 1893.

<sup>137</sup> Am. Jour. Med. Sci., 1903, vol. cxxv.

<sup>138</sup> Mitth. aus d. Grenzgeb. d. Med. et Chir., 1904, Bd. 13, p. 781

<sup>139</sup> Wien. klin. Wochenschr., 1904, No. 17.

tion of red blood-cells within the blood-stream in such numbers that the body cannot warehouse the pigment, which is therefore excreted by the kidneys. This occurs when about one-sixtieth of the total hæmoglobin is set free. It may follow various blood poisons, as potassium chlorate, pyrogallie acid, CO, naphthol, AsH<sub>3</sub>, etc.; or the poisons of fevers,—scarlet fever, typhoid, yellow fever, especially malaria, and lues; or severe burns, exposure to cold, and the transfusion of foreign serum. It may occur during pregnancy (Brauer), as an epidemic fever of the new-born, in certain cases of nephritis, and after severe intra-abdominal hemorrhages.

Curry, Brem<sup>140</sup> and others have shown that in the cases of "black water fever," which, judging from their symptoms and prompt improvement under quinine therapy, are quite certainly due to malaria, it is often not possible to find any malarial parasites either in the blood or the organs. The black water fever due to malaria may later recur after an ordinary dose of quinine.<sup>141</sup> With the hæmoglobinuria there is also an intense albuminuria. These may appear suddenly and synchronously, but the albuminuria may persist after the hæmoglobinuria has disappeared (Brem). The belief that hæmoglobinuria is the result of a hæmoglobinaemia, though probable, does not rest on a very firm basis, for, as Senator says, hæmoglobinaemia has never been proved in the hæmoglobinuria due to infectious diseases or hemorrhagic nephritis.

The PAROXYSMAL HÆMOGLOBINURIA is a condition which has attracted considerable attention. This is a rare condition of adults which occurs in attacks after exposure to cold or exertion, and which consists of hæmoglobinuria often preceded by fever and chills, and pain in the lumbar regions. The output of hæmoglobin continues for one or two days or less. The excretion is usually preceded by a hæmoglobinaemia, but in rare cases this has been missed.

Some claim that the cause is a hæmolytic action of blood-plasma (Hoover and Stone, Arch. of Int. Med., Nov., 1908, vol. ii, p. 392), others a chemical toxine, others a mechanical injury of the red blood-cells, and in the circulation shadows are found, while others think the cause is in the kidney. Senator thinks this latter is to be considered in many cases (see page 494). It is of interest that 23 of 77 cases gave a history of lues. The urine is red or dark brown; spectroscopically are found methæmoglobin alone or with hæmoglobin, microscopically are found amorphous blood-pigment in masses or casts, or even crystals of hæmatoidin; few or no red blood-cells will be found, and if present they are so few that they cannot explain the pigment; often hyaline and granular casts and renal epithelium are present; sometimes many calcium oxalate crystals also; albumin is always present, and often bile pigment, but, it is said, no bile acid. As the hæmoglobin disappears the albuminuria will continue for a short time. During the attack will be found in the blood often shadows of red blood-cells, an increase of leucocytes, amorphous masses of pigment, and a great many platelets. Sometimes

<sup>140</sup> Jour. Am. Med. Assoc., May 3, 1902; Brem, Jour. of A. M. A., Dec. 8, 1906.

<sup>141</sup> Nansen, Brit. Med. Jour., May 16, 1903.



the hæmoglobinæmia can be seen even grossly, the plasma having a reddish or a ruby-red color. It is doubtful if the isotonicity of the blood is changed. Degenerations in the red blood-cells are common, and other points indicating their lowered resistance, for instance their resistance against shaking and against  $\text{CO}_2$ . Donath<sup>142</sup> was unable to show any lowered resistance of the cells to any mechanical influence.

The immediate causes are various; among them are excessive exercise or mental excitement. Cold is the most potent, and the patient may produce it by plunging his hands into cold water. It may also be produced locally by tying a string about one finger. Homburg's patient<sup>143</sup> showed it after an involuntary cold plunge of three minutes' duration.

In hæmoglobinuria the urine may be clear, but it is usually more or less clouded by hæmoglobin casts, amorphous masses of pigment, and casts from the associated nephritis. If it be sedimented, the supernatant urine is a clear blood-colored fluid, and in the sediment so few red blood-cells that they could not possibly explain the amount of hæmoglobin. The urine must be tested fresh to determine the difference between these two conditions, since the red blood-cells will so quickly go to pieces, freeing much hæmoglobin and leaving an abundant grayish-brown albumin-rich sediment, in which may be seen the stromata of the laked red cells. Stempel reviews the literature to date in a splendid résumé.<sup>144</sup>

CHEMICAL TESTS.—These, apart from the spectroscopic, are the same for hæmoglobin and its many modifications, and whether intracellular or not. This last point can be tested only by microscopic examination.

(1) An ordinary *heat-acetic acid* albumin test is made. A brown coagulum forms which usually swims on the surface. If this be shaken with acid alcohol ( $\text{H}_2\text{SO}_4$ ), the clot is decolorized. The color depends on the amount of hæmoglobin present. This test is not very delicate.

(2) *Heller's Test*.—A test-tube is filled half-full of urine, about five drops of  $\text{NaOH}$  added to make strongly alkaline, and then warmed to form hæmatin. A brownish red or bloody precipitate results of the precipitated phosphates and carbonates of the alkaline earths which carry down the hæmatin. If the urine be already alkaline, the phosphates of the alkaline earths may already have precipitated, and hence the test fail, in which case it is necessary to add a certain amount of normal urine in order to supply these salts.

This test is very delicate, indicating 1 cc. of blood in 1 litre of urine. If the fine red blood-color of the precipitate is not evident since the urine is dark or jaundiced, the precipitate should be filtered off, and dissolved in acetic acid; a red solution is obtained which decolorizes gradually in the air. This red precipitate has by reflected light a greenish tinge. If but little hæmatin be present, the pre-

<sup>142</sup> Zeitschr. f. klin. Med., 1904.

<sup>143</sup> Ibid., vol. liii.

<sup>144</sup> Zentralbl. f. d. Grenzgeb. d. Med. et Chir., 1902, vol. v. pp. 177, 267.

precipitate should be dissolved in acetic acid and the residue of this used for the Teichmann's test. Similar red precipitates may be obtained after the ingestion of senna, rhubarb, or rhamnus. The urine, however, is yellow at first, and on the addition of the sodium hydrate becomes red. The phosphate precipitate, if dissolved in acetic acid, gives a lemon-yellow solution, which changes on exposure to the air to a violet. Hæmatoporphyrin and other pathological pigments may give a red precipitate, but the spectroscope will quickly indicate the difference by showing the alkaline hæmatin spectrum. If but a trace of blood be present the urine is first made alkaline with  $\text{NH}_4\text{OH}$  and then precipitated with tannic acid. The precipitate is used for the hæmin crystal test.

(3) *Teichmann's HCl-Hæmin Test*.—The precipitate obtained by either of the preceding tests, or, better, a tannic acid precipitate, is filtered, washed, and dried in the air. A very small granule of the dry precipitate is put on a slide with one granule of  $\text{NaCl}$  and a few drops of glacial acetic acid. The cover-glass is then put on. The specimen is then warmed over a small flame so that the acetic acid steams. The acid is constantly renewed. When the acetic acid surrounding the granule is stained a brownish color, the heating is discontinued and the slide cooled very slowly. The characteristic crystals of hæmin may soon be seen with the microscope.

The attention of teachers of clinical chemistry is directed to this test as a very good one to use as a class exercise. The application of chemical tests to specimens under the microscope, and the general subject of crystallography, are subjects of which it would seem that our laboratory workers are rather ignorant and unskilled. It is advised to give the class human blood in various conditions, and also specimens containing the blood of various animals. In order to obtain good crystals the following points must be carefully observed. The blood specimens should be thoroughly dried; only glacial acetic acid should be used; one minute crystal of sodium chloride is better than several; the acid should not boil when held over the free flame, since only slight steaming is required and further heating will spoil the specimen; the acid should be renewed in ample amounts as it evaporates during the warming; and finally the specimen should be cooled very slowly. Excellent specimens may be obtained without the use of heat if the specimen is allowed to stand for twenty-four hours.

(4) The tannic acid precipitate mentioned in test (2) may be ashed on a platinum-foil, the ash dissolved in a few drops of hot  $\text{HCl}$ , this diluted and filtered and tested with the potassium ferrocyanide solution.

(5) The *Guaiac test* (Schönbein-Almén test) is very delicate. The urine is overlaid carefully with a mixture of equal parts of Guaiac tincture (alcoholic solution of *resina Guaiaci*, 1 to 5) and old oxygenated oil of turpentine. The turpentine should be exposed to the air for some





time, that it may be well oxygenated; the Guaiac tincture should not be exposed to the sun or air, and should be kept in a colored bottle. These solutions when mixed should show no blue color. The urine is superimposed with this, and if blood be present an intense blue ring appears at the line of separation.

The urine must be acidified with acetic acid if necessary. This test is so delicate that it may be positive when the spectroscopic test is negative. Pus need not be excluded unless the above solutions have not been properly kept. The test should always be controlled with a fluid known to contain blood. The test is not absolutely positive, since other bodies will give it, yet it always has a negative value, since if negative no blood is present.

That test for blood in the urine which would appear to be the most delicate of all is the phenolphthalein test of Kastle (Bull. 51, Hygienic Lab., Public Health and Marine Hosp. Service). This is said to show 8 parts of blood in 1,000,000 parts of urine.

*Spectroscopic.*—In the spectroscopic examination of urine containing blood a mixed spectrum may usually be expected. If the blood is fresh, the lines of oxyhæmoglobin will predominate, but in hæmoglobinuria, or in nephritis, of methæmoglobin. Bacteria will oxidize the last two pigments to oxyhæmoglobin. The urine should be diluted if necessary, and must be clear.

Very small amounts of blood-pigment are detected as follows (Hoppe-Seyler): To 100 c.c. of urine is added an albumin solution or an albuminous urine. This is heated, that a good coagulum may form, the precipitate washed, pressed out, and rubbed up with alcohol which contains a little  $\text{H}_2\text{SO}_4$ . This is then warmed and filtered. The filtrate after treating with  $\text{NaOH}$  and  $(\text{NH}_4)_2\text{S}$  gives the bands of hæmatin.

**Methæmoglobin.**—Many previous observations of methæmoglobin in the urine are not reliable, since hæmatin was not excluded. Methæmoglobin is present in all fresh urines containing blood, although later it may be oxidized to oxyhæmoglobin. One should not be content to find a spectrum resembling that of neutral methæmoglobin, since this resembles the spectrum of hæmatin, but should add ammonia and demonstrate the spectrum of alkaline methæmoglobin. This spectrum may be confused if other bodies which have a spectrum or which darken the field, as bile or urobilin, are present. One must be careful not to dilute the urine too much.

Urobilin or bile-pigment may be removed with basic  $\text{PbAc}$ , the hæmoglobin remaining in solution, but methæmoglobin will be precipitated. In case red blood-cells are present, water should be added in sufficient amount to luke them. If no absorption bands are seen, or they are very faint, the hæmoglobin may be transformed to reduced hæmatin, whose spectrum it is easier to study. This reduction is best done with  $(\text{NH}_4)_2\text{S}$ , or with  $\text{NaHSO}_3$  and zinc, for only a short

time, else the albumin will be precipitated. The spectrum of reduced hæmoglobin is fainter than that of oxyhæmoglobin.

**Hæmatoporphyrin.**—Hæmatoporphyrin, an iron-free derivative of hæmoglobin, is present in normal urine in traces, but sometimes it is excreted in very large amounts. The use of sulphonal is one of the most important causes of hæmatoporphyrinuria. It is present in larger amounts, however, in cases of rheumatism, pericarditis, Addison's disease, paroxysmal hæmoglobinuria, cirrhosis of the liver, pneumonia, and hæmatemesis. Some claim that for the diagnosis of liver disease this increase is important. It is increased in lead poisoning. It is also increased in acute infectious diseases and in various forms of tuberculosis.

The color of the urine is sometimes deceptive, especially in cases due to lead poisoning. Surely it is not the hæmatoporphyrin in the urine which explains its peculiar color, since there may be considerable of this pigment present in a normally colored urine, also the hæmatoporphyrin may be removed from a highly colored urine without changing its appearance. Finally, some urines whose color strongly suggests the presence of this pigment contain none. What the pigments are which are so often excreted with hæmatoporphyrin is not known (Monro, *Quart. Jour. Med.*, 1907, vol. i, p. 49; 1910, vol. iv). The colors of the urine supposed to indicate the presence of hæmatoporphyrin are dark brownish-red, cherry-red, or Bordeaux-red, and "Port-wine" in color.

About 40 fatal cases of hæmatoporphyrinuria have been reported, chiefly in women, following the use of sulphonal. Trional and tetronal also cause it.

The cause of this hæmatoporphyrinuria is not known. Some claim that lead, sulphonal, etc., have a direct action on the red blood cells; others that they cause hemorrhages into the gastric mucosa, that the blood pigment in these is changed by the gastric juice to hæmatoporphyrin, which is absorbed and excreted. In other cases the only trouble is said to be a lesion of the renal epithelium. In hæmoglobinuria there is preceding hæmoglobinæmia, as a rule, but a hæmatoporphyrinæmia has not yet been proved.<sup>145</sup> Some ascribe the condition to perverted catabolism of hæmoglobin rather than to increased destruction of red cells.

Pal<sup>146</sup> reports a case of paroxysmal hæmatoporphyrinuria with "black" urine, with symptoms similar to those of paroxysmal hæmoglobinuria, and due, he thinks, to lues.

Garrod<sup>147</sup> collected 12 cases not due to sulphonal. Nearly all these patients were men; the condition lasted years without bad symptoms.

For detecting hæmatoporphyrin in the urine Salkowski recommends the following method. One completely precipitates from 30

<sup>145</sup> Ruedy, *Am. Jour. Insanity*, October, 1899.

<sup>146</sup> *Centralbl. f. inn. Med.*, 1903, vol. xxiv. p. 601.

<sup>147</sup> *Lancet*, March 5, 1904.

to 50 c.c. of urine with an alkaline solution of barium chloride (equal parts of a cold saturated solution of barium hydroxide and of a 10 per cent. solution of barium chloride), and filters it. The precipitate is washed—first with water, next with absolute alcohol. It is then extracted by repeatedly pouring warm acidified alcohol (10 c.c. of alcohol containing from 6 to 8 drops of HCl) over the precipitate on the filter paper. The alcoholic filtrate will be a reddish-violet solution of acid hæmatoporphyrin with a two-band spectrum, and this spectrum by the addition of ammonia (which changes the color of the solution to yellow) is transformed to the four-band spectrum of alkaline hæmatoporphyrin (Sahli).

#### SEDIMENTS.

**Preservation of the Urine.**—To study the organized sediments the urine should be examined while perfectly fresh, for casts disintegrate rapidly in some urines. The urine is best sedimented by the centrifuge, but it will sediment fairly well in a conical glass, or it may be filtered through a filter paper and the last drops examined. A drop of the sediment is drawn up into a clean pipette; the outside of the pipette is then wiped off and a drop blown onto a glass slide. In case the urine contains very much sediment this will settle in layers, the components of which will vary considerably. Such a sediment should first be well mixed or the several layers examined.

To preserve the urine from bacterial action during long sedimentation a piece of camphor, or one-fifth volume of 1 to 200 chloroform water, or one-fifth volume of saturated borax solution is used. A few drops of formalin are really best, but this may add to the sediment a component of its own. Chloroform, so valuable in the preservation of urine for chemical work, is not to be recommended. Thymol is fairly good.

To preserve sediments the urine is centrifugalized and the supernatant fluid poured off. To the sediment may then be added chloroform water to preserve crystals, or formalin to 1 to 2 per cent. to preserve casts and formed elements.

Since there are very few sediments which may be recognized beyond doubt from their appearance alone, the student should practise the microchemical tests. The reagents may be drawn under the cover by applying a piece of filter paper to the edge opposite to the drop of solution. If few casts are present the surface of a large slide is covered by the urine and no cover-glass used.

There is one peculiarity of crystalline sediments worthy of mention,—that the crystals of any one substance in any one urine usually belong to the same system. Another peculiarity is the relative infrequency of crystalline sediments in women's urine.

The sediments have been divided into the organized and unorganized. By the former are meant tissue constituents, casts, bacteria, and formed elements from the urinary or communicating organs.

The reaction of the urine may often be detected from the gross sediment; when acid this is granular, when alkaline, mucoid. Specimens should be examined when made, since if allowed to dry even a little the crystals which separate are quite confusing.

**Unorganized Sediments.** (1) **Urates and Uric Acid.**—A precipitation of urates occurs in any concentrated acid urine, especially on a cold day, much to the distress of mind of some persons. The urine first presents a very milky appearance, and the sediment then settles on the bottom and sides of the glass, forming a heavy voluminous mass. The color of the precipitate will vary from a yellow to a bright rose-red. It is soluble in acids with the subsequent precipitation of uric acid, and in alkalies. It is easily soluble on warming. While common in any concentrated scanty normal urine, it is especially so in certain fevers, chronic passive congestion, pneumonia, and rheumatism, seldom in nephritis or in albuminous urines.

One of the most remarkable crystal forms are the long branching rods like huge yellow bacteria, which disappear on warming.

This precipitate is said to be the *quadriurates* (Roberts),— $\text{MH-}\bar{\text{U}}\bar{\text{U}}$ , which are formed by the action of  $\text{MH}_2\text{PO}_4$  on the biurates,  $\text{MH}\bar{\text{U}}$ ; if in sufficient concentration they are precipitated. The quadriurates in solution are easily decomposed to biurate and uric acid, the latter precipitating as little bright red so-called *red pepper granules* on the sides of the glass. This will be the only sediment in case the quadriurate is not in sufficient concentration itself to precipitate. In the urate sediment are found also calcium oxalate crystals, and, as ammonia is soon formed, a certain amount of the acid urates will be dissolved, some will be transformed to ammonium urate, hence in the same sediment may occur ammonium urate, the so-called quadriurates, uric acid and calcium oxalate, and even a few triple phosphate crystals. This transformation occurs progressively from above downward.

This explanation of Roberts is so satisfactory that it is unfortunate that it has little evidence behind it. One thing is quite certain, that the precipitation of the urates is the result of a chemical transformation of the salt, since it precipitates in the cooling urine too slowly to be due to this alone, and warming the urine to the previous temperature does not redissolve it. Also during its formation the acidity of the urine is said to increase.

Microscopically, the acid urate sediment consists of very fine granules in clusters of a yellow to a reddish-brown color which disappear on warming. On the addition of a little acetic acid the subsequent crystallization of uric acid may be watched.



**AMMONIUM BIURATE.**—Ammonium biurate (see Fig. 32) is the only urate sediment which forms in an alkaline urine. It may also form while the urine is very faintly acid. It occurs with the acid urate sediment after the reaction changes; also with amorphous phosphates and triple phosphates. It is formed as ammonia increases in the urine. Microscopically it is a beautiful sight, the spheres often presenting the so-called “morning star” shape or “thorn-apple” crystals. These are spheres of a dark color, often concentric or radially striated, and have on their surface thorns. More often these spheres have long projections, giving them very bizarre shapes. They are soluble in acetic acid with the subsequent precipitation of uric acid, giving off ammonia.

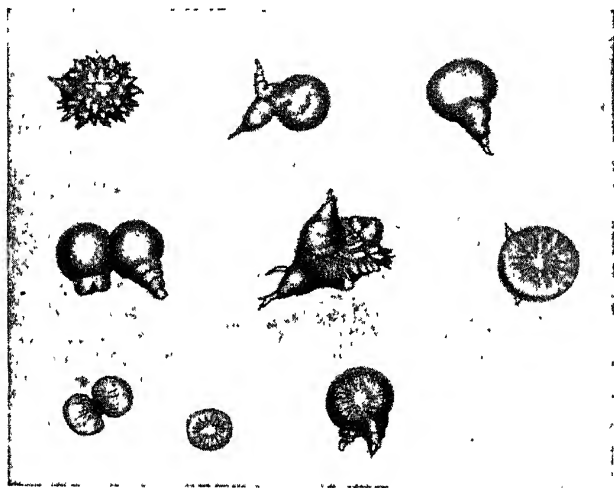


FIG. 32.—Various forms of ammonium biurate crystals.  $\times 400$ .

The yellow color of these urate sediments is due to urochrome especially, also urobilin. The red is due to uroerythrin. This sediment may contain all the bile that there is in the urine and much of the black pigment in case of carbolic acid poisoning.

Crystals of sodium biurate are rare, and occur in urines undergoing ammoniacal decomposition but yet amphoteric. These crystals resemble calcium phosphate, but are soluble in acetic acid, which gives at once a cloud of uric acid crystals.

**URIC ACID.**—Uric acid (see Fig. 34) when pure crystallizes usually in rhombs, but in the urine the corners are dissolved, giving the so-called “whetstone” crystals. When seen on the edge these crystals are very narrow rectangles. They may be single or in rosettes, or clustered in the shape of a barrel (see Fig. 34, a, b, 1). Their color is from a yellow, to a brown, or they may be colorless. The colorless crystals are sometimes perfect hexagons (see Fig. 42, e), in which case

their recognition is difficult, since they resemble cystin perfectly. A recent case of Dr. Fletcher's, the urine of which he kindly gave me for demonstration, illustrates this point. The patient was a girl six years of age, with diabetes mellitus. The urine was 1000 to 2000 cc. in amount, specific gravity about 1035, and sugar 5.1 to 5.5 per cent.; nothing of interest microscopically. After twenty-four hours on a carbohydrate-free diet the urine was turbid, showing a suspension of glistening particles (sp. gr. 1026; sugar 0.6 per cent.). Microscopically, the turbidity was seen to be due to colorless hexagonal crystals almost exactly resembling cystin, many single, most in clumps of even macroscopic size. It was only after testing them chemically that they were recognized to be uric acid. On this day no typical uric acid crystals were seen. The following day there was a mixture of hexagonal and whetstone crystals, and later none of the former were found.

Some are in needles arranged in sheaves (see Fig. 34, 4).

Their color is due to urochrome, not to urobilin, and the red is due to uroerythrin plus urochrome. Urobilin, hæmatoporphyrin, bilirubin, or biliverdin may give the color to the crystals. In cases of carbolic acid poisoning these crystals are a dark brown, almost black color. These crystals may occur in masses as large as the head of a pin, which cling to the glass (see Fig. 34, 2).

If these crystals are precipitated artificially by acid they are of a reddish-brown color due to black decomposition products of urochrome; they may be stained by indigo-blue or indigo-red.

CALCIUM URATE crystals are said to sometimes occur with calcium oxalate; they are colorless prismatic crystals, insoluble in hot water, give the murexid test, and if acid be added uric acid crystals are deposited. They may be produced by treating the urate sediment with lime water.

DETECTION.—In acid urine the urate sediments may usually be recognized from their gross appearance, but particularly from the fact that they disappear on warming and that all are dissolved by acid with the subsequent precipitation of uric acid. Uric acid itself is not dissolved by heat or acid. Ammonium biurate is soluble in acid, and uric acid crystals then appear. The spheres are of characteristic form.

MUREXID TEST.—The crystal or sediment is evaporated in a porcelain dish with dilute  $\text{HNO}_3$ . To the residue is added weak  $\text{NH}_4\text{OH}$ . A beautiful purple-red color is obtained.

The significance of the urate sediment is very slight, since it depends chiefly on the concentration and the acidity of the urine. Uric acid,

FIG. 33.—Sheaves of ammonium urate (?) needles.  $\times 50$ .



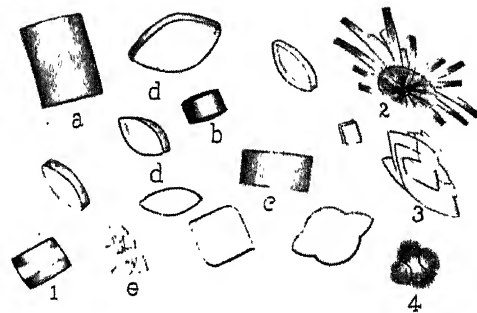


FIG. 34.—Uric acid crystals. (The lettered forms are drawn from nature, the figures copied from Rieder's Atlas.)



however, is somewhat more important since it may form large concretions.

**Phosphates and Carbonates.**—(1) AMORPHOUS EARTHY PHOSPHATES AND CARBONATES may be precipitated in any urine by the addition of a little fixed alkali. A somewhat similar precipitate forms when a weakly acid or alkaline urine is heated, since the acid salts are changed to insoluble basic salts. Both are soluble in acetic acid, the carbonates with gas evolution. They are the chief constituent of the sediment of an alkaline urine, and may cloud even the fresh urine of cases of hypersecretion who lose much acid from the stomach, from vomiting or lavage, or diarrhoea. In the so-called phosphaturia, however, the total amount of phosphoric acid is not increased. Microscopically, this precipitate appears as very coarse colorless granules varying considerably in size, which disappear on the addition of a little acetic acid. By the gas formed it may be seen which granules were carbonates.

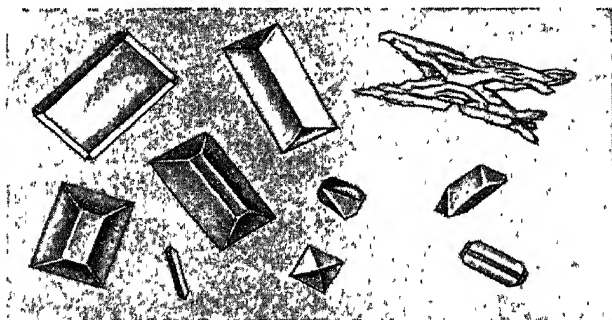


FIG. 35.—Various forms of triple phosphate crystals.  $\times 400$ . To the left are coffin-lid shapes; in the lower centre a perfect pyramid; that in the upper left corner resembles neutral magnesium phosphate; that in the upper right is a partially dissolved crystal

(2) TRIPLE PHOSPHATES,  $\text{MgNH}_4\text{PO}_4 \cdot 6\text{H}_2\text{O}$ .—These beautiful crystals (see Fig. 35) appear in urine even still acid as soon as sufficient ammonia is present to form them. They accompany usually the amorphous carbonates and phosphates, and often ammonium urate, and may be the chief constituent of the sediment. They belong to the rhombic system, and vary in size from those very small to some even 9 mm. in length. Of their shapes, the so-called coffin-lid crystals are characteristic (see Fig. 35). They are often very irregular and of a great variety of shapes, due to rapid crystallization from a concentrated solution, or especially as they become partially dissolved, leaving X-forms. Some are said to resemble calcium oxalate crystals, but we doubt this, since even when perfect pyramids with square base the difference is apparent (see Fig. 35).

Fern-shaped crystals occur especially in sediments artificially precipitated.

In some urines these crystals are nearly all of unusual shapes,—very thin plates (see Fig. 35), some with bevelled edges, some apparently not; some with square, others with rounded or bevelled corners; some are wedges (see Fig. 36), some triangular prisms; yet all give by refraction a greenish hue which is not seen in the calcium oxalate.

**NEUTRAL MAGNESIUM PHOSPHATE**,  $\text{Mg}_3(\text{PO}_4)_2 \cdot 22\text{H}_2\text{O}$ .—These very rare crystals (see Fig. 42, b) occur in alkaline urines in which not sufficient ammonia is present to form the above. Such is the case in certain cases of dilated stomach with considerable vomiting, and also after the ingestion of magnesium carbonate, etc. These crystals are exceedingly refractile, long rhombic tablets with bevelled edges. They form a beautiful sediment. Some resemble the very thin coffin-lid triple phosphate crystals (see Fig. 35).

**DICALCIUM PHOSPHATE**.—These crystals form in amphoteric or weakly acid urine. They are rare. They appear as small prisms or

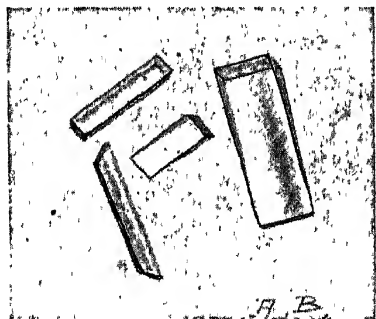


FIG. 36.—Atypical forms of triple phosphate crystals.  $\times 400$ .

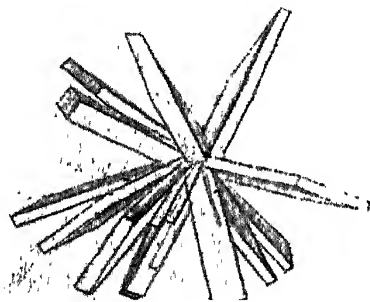


FIG. 37.—Wedges of dicalcium phosphate.

wedges in irregular clumps (see Fig. 37), or are massed together in rosettes (see Fig. 42, d) or fan-shaped clusters. These masses or rosettes are usually so thick that the individual small crystal can hardly be made out. A rather unusual form of probably calcium phosphate is shown in Fig. 38. They occur when the urine is rich in calcium and only weakly acid; among diseases, especially in joint troubles. They are soluble in acetic acid. They may be separated from triple phosphates, since 20 per cent. ammonium carbonate will dissolve these and not the latter. An unusual calcium phosphate sediment is pictured in Fig. 33.

**CALCIUM CARBONATE**.—These crystals (see Fig. 39) may be mingled with the amorphous carbonates in an alkaline urine. They occur as amorphous masses or as dumb-bells a little like  $\text{CaOx}$ , or large concentric radiating spheres. They are soluble in acetic acid with gas formation.



NEUTRAL CALCIUM PHOSPHATE also forms a scum on the surface of the urine, even when quite fresh, giving the appearance of a film of oil, and which may be easily skimmed off. This consists of an amorphous precipitate which under the microscope resembles sheets, often seen when one is not careful always to wipe off the outside of a pipette before making a preparation for microscopic examination.

**Oxaluria.**—This symptom complex, formerly so respected, has fallen into disrepute. The old criterion for its presence was a large sediment of CaOx crystals, but this sedimentation does not depend so much on the total amount of oxalic acid present as it does on its solubility. Yet it is of much practical importance, since CaOx occurs so often in calculi, in even 30 to 50 per cent. of them, and these are the worst stones. The chief source of the CaOx is the food, certain vegetables, as beans, artichokes, beets, potatoes, and especially to-



FIG. 38 —Calcium phosphate (?).  $\times 400$ .

matoes, spinach, rhubarb, certain fruits and grains, cocoa, tea, coffee being particularly rich. The most ingested is destroyed in the intestine, only 15 per cent. of the oxalic acid being absorbed; this is dissolved by the HCl in the stomach and excreted quantitatively as CaOx; about 10 per cent. is in the stools, the rest is destroyed by the intestinal bacteria and ferments.<sup>148</sup>

In health the output is about 20 mg. per day, with an upper limit of 35 mg. Although the most comes from the food, yet a certain amount is from tissue combustion, since some is present even in the urine of a starving person. Bakhoven thinks that of the foods the carbohydrates are the chief builders. It bears no relation to the uric acid excretion; the latter, for instance, can be increased by the nucleins, which do not affect the oxalic acid output.

Among the diseases claimed to be accompanied by oxaluria are pulmonary tuberculosis, peritoneal tuberculosis, pernicious anæmia, leu-

<sup>148</sup> Klemperer and Tritschler, Berl. klin. Wochenschr., 1901, p. 1289.

kæmia, in which condition the output is claimed to be 33.2 to 53 mg. per day, jaundice, diabetes mellitus, gout, diseases of the digestive and respiratory organs, cirrhosis of the liver, and especially neurasthenia. It bears some relation to the absence of HCl in the gastric juice and to fermentation processes in the intestine. In diabetes mellitus a large output is quite surely present. This increases as the sugar diminishes (vicarious oxaluria). Naunyn mentions three cases with quantitative estimations, one with 0.8 gm., the second 1.2 gms. in twenty-four hours, the third with 0.5 gm. per litre. In the case of neurotic persons an increased output is generally granted.

It is of interest that insurance companies now regard "oxaluria" as an early sign of nephritis. The best recent work on this subject is that of Serkowski and Mozdzenski,\* who showed by accurate methods that there is no apparent relationship between the amount of oxalic acid in solution and that in the sediment, or between the amount of



FIG. 39.—Calcium carbonate.  $\times 400$ .

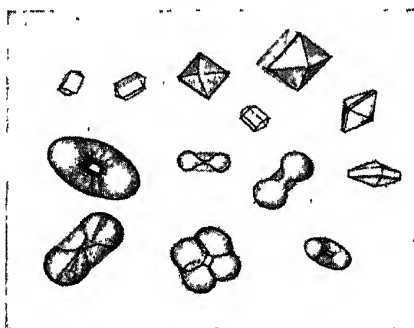


FIG. 40.—Various forms of calcium oxalate crystals and spheres.  $\times 400$ .

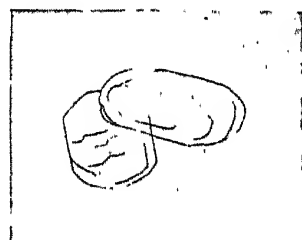


FIG. 41.—A rare form of calcium oxalate crystals.

oxalic acid and of uric acid in the urine. Its amount does run roughly parallel to that of the acid phosphates.

CALCIUM OXALATE CRYSTALS may precipitate in any urine. The cause is not fully known, but their increased presence it is hard to associate with any pathological condition. This precipitation is most important clinically. The real question is, Why is any in solution? Klemperer and Tritschler<sup>149</sup> consider all the acid phosphates aid in holding it in solution, the salts of sodium least, calcium more, magnesium most, and something depends on the absolute amount of CaOx. The crystals occur in two forms:

(1) The octahedral, which belong to the tetragonal system (see Fig. 40). These resemble double envelopes or prisms, and may be recognized from their appearance ( $\text{CaC}_2\text{O}_4 \cdot \frac{1}{2}\text{H}_2\text{O}$ ).

\* Zeitsch. f. phys. Chem., Jan., 1911, vol. lxx, p. 264.

<sup>149</sup> Zeitschr. f. klin. Med., 1902, vol. xlv, p. 337.

(2) Spheroidal forms (see Fig. 40) which are flat, oval, or nearly semicircular with a central groove; hence they resemble an hour-glass. They often present a radial striation ( $\text{CaC}_2\text{O}_4\cdot\text{H}_2\text{O}$ ).

A rare form of crystal is represented in Fig. 41, flat plates with parallel sides and rounded ends, which look like superimposed sheets of mica. In a recent case the urine had a great many of these.

These crystals are usually colorless, but may be bile-stained. They are transparent and very refractive. They are insoluble in water, very little if any in acetic acid, but easily in any mineral acid. Their crystallization probably depends on the amount of oxalic acid, on the relative amount of  $\text{NaH}_2\text{PO}_4$  which has a greater ability in holding  $\text{CaOx}$  in solution in a warm than in a cold urine, and especially in inverse proportion to the amount of magnesium. As the precipitate forms very slowly, perfect crystals form. They may be found in acid, amphoteric, or weakly alkaline urine, and are sometimes present in the specimen when voided.

They attracted considerable attention among the older pathologists, as they were supposed to cause an irritation which explained many of the symptoms and vicious habits of neurotic individuals. The shape of the octahedral forms is quite characteristic, and these cannot well be mistaken. Apart from their shape, their refractivity is very suggestive, and it is only on hasty examination that they could be mistaken for triple phosphate crystals, even when the latter are square and perfect, but single, pyramids. They may also be easily separated from these by their insolubility in acetic acid. The spherical forms could be mistaken for  $\text{CaCO}_3$ , but these are soluble in acid with gas production and show a different structure.

**QUANTITATIVE DETERMINATION OF OXALIC ACID.**—The Neumann's method is as follows: The twenty-four hours' amount of urine is precipitated with calcium chloride and ammonia, and then acetic acid is added until a weak acid reaction. A small amount of alcohol thymol solution is then added to inhibit bacterial growth. The precipitate after long standing, over twenty-four hours in a warm place, is washed several times by decantation, pouring the fluid through the filter, then the precipitate brought onto the paper. Wash as much as possible by decantation, since the fine precipitate easily passes through the paper. The precipitate is then dissolved in somewhat warmed dilute  $\text{HCl}$ , and the paper washed with water until the acid reaction disappears. The filtrate is evaporated in a porcelain dish on the water-bath to a small volume. The fluid is then placed in a small stout cylinder, the dish being washed with water and dilute  $\text{HCl}$ , and the wash-water added to the fluid. Ammonia is then added in excess and the whole stained with a few drops of litmus, to be sure of the reaction. After long standing, at least twenty-four hours, the precipitate is brought onto an ashless filter paper. It is necessary to remove the crystals from the walls of the cylinder by rubbing well with a glass rod protected with a small piece of rubber tubing. The precipitate is then washed with water until it is chlorine-free, and then with acetic acid. The filter is then dried, burned in a platinum crucible at a dull red, then heated with a blast flame until at constant weight. Calcium oxalate is thus transformed to calcium oxide, 50 parts of which correspond to 90 parts of oxalic acid.

**CALCIUM SULPHATE,  $\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$ .**—This is a very rare sediment occurring in very acid urines. The crystals (see Fig. 42, a) are long and thin tablets or needles, single, but more often in clusters, which are insoluble in  $\text{NH}_4\text{OH}$ , alcohol, and acetic acid. They are difficultly soluble in  $\text{HCl}$ ,  $\text{HNO}_3$ , and hot water. They are more soluble in hot water than in cold. The solution should be tested with  $\text{BaCl}_2$ , to make sure of sulphuric acid.

**Hippuric Acid.**—This acid occurs rarely as a sediment, as milk-white, semi-transparent, four-sided prisms and rods with ends of two to four planes (see Fig. 42, c). These are distinguished from uric acid, which they may resemble in form, by their greater solubility in water, especially in warm, their solubility in alcohol and ether, and that they do not give the murexid test. The normal amount of hippuric acid in the urine is from 0.1 to 1 gm. per day, and varies as the diet.

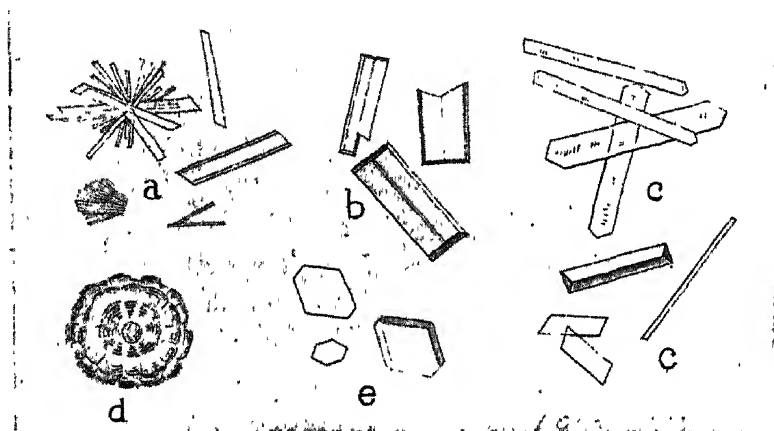


FIG. 42.—Various crystals. a, calcium sulphate; b, neutral magnesium phosphate; c, hippuric acid; d, acid calcium phosphate; e, colorless uric acid. (Copied from Rieder's Atlas.)

**Hetero-albumose.**—In two cases it has been found in the sediment, once crystalline and once amorphous.

**Xanthin.**—Two or three cases have been reported in which xanthin crystals appeared in the sediment. These resembled uric acid somewhat (see Fig. 43, d), but are soluble on heating and in ammonia. They are evaporated in quite concentrated  $\text{HNO}_3$  on a bath, and give a yellow residue. On careful heating further over a small flame this becomes intensely yellow, and if  $\text{KOH}$  be added, yellowish-red. Warmed further, it becomes a deeper red, even a violet-red. This is not the murexid test, and should not be confused with it.

**Hæmatoidin (Bilirubin).**—These crystals appear as needles (Fig. 43, a) or rhombs (b) in the sediment, sometimes in hæmorrhagic nephritis, and in very jaundiced urine especially after acid is added, in acute yellow atrophy and in fragments from cancers. They also occur in pyonephrosis and after transfusion. In the jaundice of the newborn they occur in the epithelial cells of the urine. They have also been found in waxy kidney, scarlet fever, typhoid fever, and carcinoma of the liver with jaundice. They also occur in amorphous form.

**Indigo.**—The crystals of indigo may occur in normal decomposing urine as a scum of blue needles arranged in stars, or blue rhombic plates, soluble in chloroform to blue solution. These are more often seen in the decomposing urine of peritonitis, pyelonephritis, etc. One also sees violet-red bundles of crystals or plates of indigo-red.

**Melanin** has been found rarely as amorphous scales.

**Hæmoglobin** occurs in cases of hæmoglobinuria as amorphous scales, plates, or casts.

**Cholesterin.**—Cholesterin sometimes occurs in flat superimposed plates, often with re-entrant angles (see p. 708), in such amounts as to justify the term “cholesterinuria.” It is always found in association with other fats. This may occur in vesical catarrh, especially in pyelitis, pyonephrosis, echinococcus cysts of the kidney, and nephritis. The crystals are also formed from the fatty degeneration of pus-cells and of breaking-down tissue. It is rare from fatty degeneration of the kidneys, and does not occur in chyluria, in which case one would

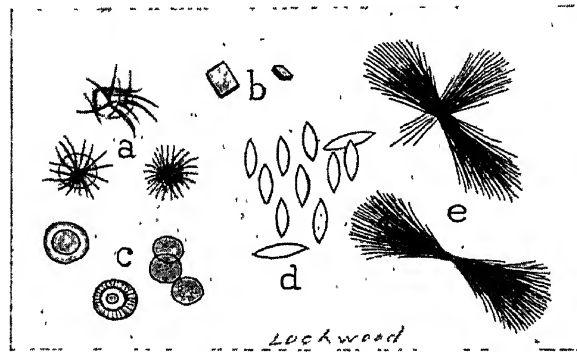


FIG. 43.—Various crystals of the urine. a, hæmatoidin needles; b, hæmatoidin crystals; c, leucin, d, xanthin; e, tyrosin. (Copied from various authors.)

expect it. Hirschlaff<sup>150</sup> reports a case of hydronephrosis (thought to be due to a stone and with the emptying of a large sack) with even 5.8 gms. of cholesterin in 100 cc. of urine. We had a case of long standing cholesterinuria of considerable degree in a case of renal cyst of doubtful nature.

Cholesterin is insoluble in cold alcohol, but easily in hot, reprecipitating on cooling, and is soluble in chloroform. If the cholesterin solution be superimposed on concentrated  $H_2SO_4$ , the former solution is first blood-red, then more violet-red, while the sulphuric acid becomes dark red with green fluorescence (Salkowski). If the crystals microscopically be brought into contact with  $H_2SO_4$  4 parts,  $H_2O$  1 part, this play of colors can be watched.

**Leucin and Tyrosin.**—Leucin and tyrosin occur in the urine in certain pathological conditions. As a spontaneous sediment leucin does not occur, while tyrosin has been found in very few (three) cases, one

<sup>150</sup> Deutsches Arch. f. klin. Med., 1899, vol. lxii. p. 531.

of which was of acute yellow atrophy, one of phosphorus poisoning. These bodies may often be found in solution in acute yellow atrophy, phosphorus poisoning, rarely in smallpox, severe typhoid fever, pernicious anæmia, leukæmia and sometimes in cases with simple cardiac liver (Mann, Quart. Jour. Med., October, 1907). It is as a rule necessary to evaporate the urine to about one-tenth its volume. The addition, then, of alcohol will usually give a sedimentation of needles of tyrosin and spheres of leucin; peptone and lactic acid are also present. The needles of tyrosin are black in appearance and are grouped together like sheaves of wheat (see Fig. 43, e). Since jaundice also occurs in practically all cases, the crystals of bilirubin must be excluded; these have an intense brown color, but in some cases a rather similar shape. In an alkaline urine the calcium phosphate must be excluded. LEUCIN, if pure, is in groups of spherules (see Fig. 43, c) which have little refractility and hence differ from the urates. They have a much clearer contour and no spicules, and a hyaline or a radiating structure. Their appearance varies, however, with their purity, and if impure they may be in spheres or masses with no hyaline structure whatever. They may have a dark centre and a clear periphery, or *vice versa*.

The microscopical diagnosis of these bodies is almost never sufficient, but should be confirmed by chemical tests. In so doing it is quite necessary to use a fresh urine, since these bodies rapidly and easily form in a decomposing albuminous urine, hence in an old urine the question is whether they are preformed or not.

In all tests it is necessary, first, to remove the albumin by heat and acid and examine the filtrate. This is first precipitated with neutral, then with basic, lead acetate until all precipitation ceases. The urine is then filtered, the lead removed with  $H_2S$ , the filtrate concentrated by evaporation. The tyrosin even now separates out slowly if in considerable amount. The concentration should be carried on to very small volume, and the urea extracted by absolute alcohol. The residue is then boiled with weak ammoniacal alcohol and the filtrate is again evaporated to small volume and then allowed to crystallize. The leucin or the tyrosin will separate out, that one first which first becomes saturated. The partial separation may be obtained with alcohol in small volume which dissolves the leucin more easily than the tyrosin. If no precipitate appears, again dilute and precipitate with basic lead acetate and repeat.

A better separation of leucin and tyrosin is the following. The residue after evaporation is dissolved in boiling water plus a little ammonia. To the hot solution is added basic lead acetate, stirring all the while until the precipitate is no longer brown but white. It is then filtered, heated nearly to boiling, made slightly acid with dilute  $H_2SO_4$ , and then boiled to drive off the ammonia and to pre-

precipitate the lead. It is then rapidly filtered and cooled. The tyrosin will precipitate almost quantitatively. To the solution is added  $\text{H}_2\text{S}$  to precipitate the lead, and it is evaporated to smaller volume. While boiling  $\text{Cu}(\text{OH})_2$  freshly precipitated is added in excess and the boiling continued for a few minutes. The precipitate will contain part of the leucin. This precipitate is suspended in boiling water, decomposed with  $\text{H}_2\text{S}$ , and a little acetic acid added. It is then filtered. The filtrate is decolorized with animal charcoal and evaporated to small volume. On cooling the leucin will separate out. The rest of this body will be in the blue copper compound. It is very hard to get leucin pure, although it can be done by forming its ethyl ester.

**Tyrosin**,  $\text{C}_6\text{H}_4\text{CH}_2\text{CHNH}_2\text{COOH}$ .—Tyrosin crystals (see Fig. 43, e) precipitate from water solutions in bundles of needles arranged like sheaves of wheat, from ammoniacal alcohol in bunches of prisms. These are soluble in water, slightly in alcohol, not at all in ether, and easily in acids and alkalis. Its crystalline shapes are not characteristic. The sediment should be filtered out, washed with water, dissolved with ammonia plus a little ammonium carbonate in warm solution, and evaporated until it recrystallizes. The chemical tests cannot be made directly in the urine.

**PIRIA'S TEST**.—Some dry tyrosin is placed in a test-tube and a few drops of concentrated  $\text{H}_2\text{SO}_4$  added. This is warmed gently and then boiled in a water-bath for half an hour. A red solution of tyrosin sulphate is obtained. The solution is cooled. To it should be added several volumes of water. This fluid is then neutralized with  $\text{BaCO}_3$ , and filtered. The filtrate is evaporated to a few cubic centimetres, and weak  $\text{Fe}_2\text{Cl}_6$  is then added (acid-free) to the cooled solution. A fine violet color results. This test is prevented by free mineral acids or an excess of  $\text{Fe}_2\text{Cl}_6$ .

The hot aqueous solution of tyrosin gives, with Millon's reagent,  $(\text{Hg}(\text{NO}_3)_2 + \text{KNO}_2)$ , while hot a fine red color, and an abundant red precipitate.

**Leucin**  $(\text{CH}_3)_2\text{CHCH}_2\text{CHNH}_2\text{COOH}$ .—Leucin is present as spherules; their color and regularity of outline depend on the purity of the specimen. Often daughter spherules project from them, and they frequently show a striation. Leucin (see Fig. 43, c) is soluble in water, less in alcohol, and very in acids and alkalis. All of these compounds are more soluble in an impure than in a pure condition. None should ever be expected in a sediment until the urine is concentrated. It is isolated by the above methods. For the chemical tests it must first be purified by recrystallizing from hot ammoniacal alcohol. The characteristic tests are its crystallized form when pure, the fact that it sublimes at a gentle heat at  $170^\circ \text{C}$ . without fusion to a woolly mass and with the odor of amylamine.

**SCHERER'S TEST**.—Pure leucin plus a little  $\text{HNO}_3$  is evaporated on a platinum foil. A colored residue is obtained. The slight residue is warmed with  $\text{NaOH}$ , and a water-clear, if pure, or colored if im-

pure, fluid results. This is evaporated carefully and an oily fluid obtained which rolls around without wetting the foil. This test is characteristic for even impure leucin.

**SALKOWSKI'S TEST.**—To the specimen is added a little water plus one or two drops of 10 per cent.  $\text{CuSO}_4$ . A blue solution is obtained,  $(\text{C}_6\text{H}_{12}\text{NO}_2)_2\text{Cu}$ , which does not reduce on heating.

**Cystin.**—This rare condition (only 131 cases now reported in literature. Simon and Campbell), which occurs in certain persons perhaps during their whole life, is accompanied by no symptoms except those from the calculi formed; some persons undergo repeated operations because of these stones, and live a life of misery. The formation of calculi, however, is intermittent, and after a period of misery the

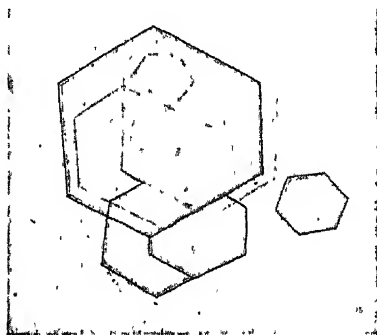


FIG. 44.—Cystin crystals from urine.  $\times 400$ .

person may for a long time be free. The output of cystin is in some cases intermittent.

Cystin is a normal intermediate product of normal proteid metabolism, the sulphur portion of the proteid being for the most part, possibly all, in this radical. It is not normal in the urine; if fed to a normal person, about 66 per cent. of its sulphur is excreted as sulphates and about one-third as neutral sulphur; none as cystin. Simon and Campbell<sup>151</sup> think some is eliminated in the bile as taurocholic acid. Why it should be excreted is not known. There are two general theories,—the one that it is the product of an intestinal mycosis, which is borne out by the fact that both intestinal contents and urine contain certain diamines, as cadaverin, putrescin, and others; the other, that it is an individual variation in metabolism, an inability on the part of the organism to oxidize the cystin nucleus.

In the urine while fresh are seen hexagonal transparent crystals of cystin. These crystals (see Fig. 44) are very characteristic, yet not absolutely so, since in certain cases the uric acid may assume this form. Sometimes large concretions form, from a pin-head size to 1 cm. in diameter, which are rather soft and waxy, crystalline on cross-section, and are of a whitish-yellow color. These crystals are soluble in ammonia and reprecipitated by acetic acid, a test which must be applied to exclude uric acid.

<sup>151</sup> Johns Hopkins Hosp. Bull., 1904.



We have seen but four cases. One of these patients has on many occasions for some years had these stones crushed. Another case, a woman, was distressed for years by these concretions, but refused operation, and as she has since then attained considerable success in public life, we presume that the stones no longer bother her so much.

The urine in such cases on standing often gives the odor of  $\text{H}_2\text{S}$ . It is in this condition particularly that the neutral sulphur of the urine is largely increased, and the neutral sulphur is the best index of the amount of cystin present.

The presence of **diamines** in the urine and in the fæces in traces has attracted some attention.<sup>152</sup>

There occurs sometimes putrescin, sometimes cadaverin, sometimes both, and their presence is variable and intermittent. Lewis and Simon, in 1902, stated that they had been found in seven cases.

Baumann's method for their detection is as follows: The twenty-four hour amount of urine is shaken up with 10 per cent. NaOH and benzoylchloride (in the proportion of 1500:200:25) until the odor of benzoylchloride is gone.

The precipitate (of phosphates, carbohydrates, and benzoylated diamines) is filtered with the aid of a suction-pump. The precipitate is digested with alcohol, filtered, the extract evaporated to small volume, 30 volumes of water added, and allowed to stand twelve to forty-eight hours. The benzoylated diamines separate out in the milky fluid as a voluminous sediment of white crystals. This is redissolved in alcohol, concentrated to small volume, and diluted again with water. This is repeated several times to separate the carbohydrates.

From the first filtrate more may be recovered by acidifying with  $\text{H}_2\text{SO}_4$  and extracting three times with ether. To the ether residue is added 12 per cent. NaOH till neutral, then 3 to 4 volumes of the alkali. This is then kept in a cold place for crystallization and crystals of cystin and the diamines will separate. They are filtered and suspended in cold water; the benzoylchloride crystals remain.

The crystals are dissolved in a little warm alcohol, then 20 volumes of ether added; benzoylputrescin is precipitated, melting point  $175^\circ$  to  $176^\circ$  C. The ether residue contains benzoylcadaverin, melting point  $129^\circ$  to  $130^\circ$  C.

**Unorganized Sediments.**—The following outline for use in recognizing an unorganized sediment is so useful that we quote it in full as given by Neubauer and Vogel.

A. Acid urine.

(a) Sediment amorphous.

(1) Sediment consists of fine granules in clumps, mingled with which are crystals of uric acid and calcium oxalate; *urate sediment*. This sediment is soluble on warming, and if a drop of strong acetic acid be added the granules gradually disappear with the subsequent separation in a few hours of uric acid crystals.

(2) Dumb-bell shaped bodies.

(a') Insoluble in strong acetic acid, soluble in concentrated hydrochloric acid without subsequent crystallization; *calcium oxalate*.

<sup>152</sup> Simon, Am. Jour. Med. Sci., 1900, vol. cxix. p. 39; 1902, vol. cxxiii. p. 838; Schollberg and Garrod, Lancet, August 24, 1901.

(b') Insoluble in concentrated hydrochloric acid; probably *calcium sulphate*. The sediment should be filtered, washed, dissolved in much hot water, and tested for calcium and sulphuric acid.

(3) Very refractive globules, soluble in ether; *fat*.

(4) Amorphous yellow granular masses: bilirubin or *hæmatoidin*.

(b) Sediment crystalline.

(1) Yellow or brown whetstone-shaped crystals, single or rosettes, alone or with amorphous urates and calcium oxalate: *uric acid*. These crystals are soluble in sodium hydroxide, then with the addition of concentrated hydrochloric acid a reprecipitation of uric acid crystals.

(2) Small yellow rhombic tablets alone or with amorphous granular tablets of the same color, often embedded in tissue detritus: *bilirubin* or *hæmatoidin*.

(3) Colorless (or yellow in a decomposed urine), transparent, strongly refractive octahedrons, or double envelope forms, or quadrangular short and narrow prisms with octahedrons at the ends, insoluble in acetic acid, soluble in hydrochloric acid: *calcium oxalate*.

(4) Crystals somewhat similar to the last mentioned, or large coffin-lid crystals, soluble in acetic acid: *ammonium magnesium phosphate* (*triple phosphates*).

(5) Symmetrical hexagonal tablets, sides and angle almost equal, insoluble in acetic acid, soluble in ammonia: *cystin*.

(6) Colorless whetstone-shaped tablets, insoluble in acetic acid; soluble in ammonia. On the addition of hydrochloric acid to this solution hexagonal tablets separate: *xanthin*.

(7) Large, flat, strongly refractive elongated rhombic tablets, soluble in acetic acid, and partially in ammonium carbonate: *normal magnesium phosphate*.

(8) Prisms, single or in rosettes,

(a') Soluble in ammonia: *hippuric acid*.

(b') Insoluble in ammonia and in acids: *calcium sulphate*.

(9) Wedge-shaped prisms, single or in clusters, or in thick rosettes, which are decomposed by ammonium carbonate, and soluble in acetic acid: *acid calcium phosphate*.

(10) Bunches of very fine needles insoluble in acetic acid, soluble in ammonia and hydrochloric acid: *tyrosin*.

B. The urine alkaline when the crystal precipitates. (After the urine becomes alkaline many of the sediments previously mentioned which separate in the acid urine may still remain.)

Amorphous.

(1) Small granules together with triple phosphate crystals,

(a') Soluble in acetic acid without gas formation: *normal phosphates of the alkaline earths*.

(b') Soluble, but with gas formation: *carbonates of the alkaline earths*.

(2) Dumb-bell shaped masses or large spheres, soluble in acetic acid with gas formation: *calcium carbonate*.

(3) Large dark spheres often covered by small projecting crystals: *ammonium urate*, soluble in hydrochloric acid or acetic acid with the subsequent separation of the rhombic crystals of uric acid.

Crystalline.

(1) Large colorless prisms, many coffin-lid shaped: *triple phosphates*, soluble easily in acetic acid.

(2) Rosettes of very fine blue needles or blue tablets: *indigo*.

(3) Rosettes of violet-red needles or rhombic platelets: *indigo-red*.

**Chyluria.**—Chyluria differs from lipuria in its gross characteristics, the term being used of a urine which resembles an emulsion of fat, hence like dilute milk. When less fat is present and the gross appearance not so striking, the term *lipuria* is used.

In chyluria considerable fat is present. This may form gross talloil-like masses, but as a rule the particles are microscopically much finer than in milk, even on the limits of visibility. The urine appears like a thin milk, and sometimes has a reddish tinge of blood, in other cases a whey-like appearance. Fresh, the urine is weakly acid or neutral, and does not have the normal urinary odor. On standing often a cream arises or a fibrin coagulum forms. In addition to the fat the urine contains always albumin, sometimes cholesterin and lecithin. The proteids found are serum globulin and albumin. Fibrogenic substances have also been found, hemialbumose and peptone. The proteid may be present from 0.2 to 2 per cent. or more, and the fat from a trace to 3 per cent. A few leucocytes may be present and a few red blood-cells. In parasitic chyluria one finds the eggs and embryos of *Filaria* usually in coagula. Casts, etc., are always absent unless a complicating Bright's disease is present. The urine may be chylous during the night, and clear during the day, or *vice versa*. In other cases the excretion of the fat is dependent upon the position of the patient, occurring only when he is in a vertical position, after digestion, bodily exercise, or excitement. In certain cases the coagula formed in the bladder have caused considerable trouble. It is a disease which lasts from months to years, often with intermissions. It may cease spontaneously. This disease occurs endemic in the tropical and subtropical regions, in some cases in the temperate zone.

There are two forms, that due to the *filaria*, and the non-parasitic form the etiology of which is not understood.

Concerning the latter, some say that sugar is not present in the urine, and were it simply lymph present it certainly would be; also

that there is a higher percentage of fat in the urine than occurs in the lymph. Again, there is no decrease in the percentage of the normal urine constituents. In some cases a fat diet will increase the chyluria, and even a foreign fat may be recognized. The theory of Claude Bernhard was that chyluria was the result of an abnormal fat content of the blood due to poor assimilation; but an increase of the fat of the blood is very rare, and this does not explain the albumin found in the urine. Others think it due to liver disease. All that can be said is that there is no severe renal lesion which explains it. Franz and Styskal think that the fat escapes from the lymphatic vessels since the chyluria diminishes or disappears if the patient be fed a fat-free diet or starved; since foreign fats of the food can be recognized in the urine; and, finally, since the cells of this exudate are lymphocytes.

**LIPURIA.**—As has been said above, this differs from chyluria in its gross appearance. It is a condition which is often reported in the hospitals, but by beginners who have not excluded oil in the catheterized specimens of urine. Again, the microscopical appearance is not always sufficient. It should be tested chemically, the urine extracted with ether, and the residue examined. This heated gives the odor of acrolein. The residue also will make a fat-spot on paper, and will give the osmic acid test.

Also to be excluded are, fat from the rectum, deception, the tenacious phosphate sediment, and the scum of bacteria forming at the top of the urine. Normally there is microscopically little if any fat in the urine, and of this the source is the blood. Lipuria may result from an over-ingestion of fat in the diet or as a medicine (cod-liver oil), the so-called "alimentary lipuria;" from the subcutaneous injection of oil or oil rubbed into the skin; the fat may come from various organs, especially after fractures of bones if the marrow be crushed; rarely after inflammation of the marrow; in eclampsia, which disease was formerly supposed to be due to the crushing of the fat of the pelvis of the kidney; crushing or tearing of the subcutaneous fat, of the liver, or of fatty tumors; among diseases are, diabetes mellitus, alcoholism, tuberculosis, adiposity, nephritis, certain mental diseases, pancreatic diseases, cardiac diseases; after various protoplasmic poisons. In the last mentioned group there may be an increase of fat in the blood, but this needs confirmation. The relation of the lipuria to lipæmia has been proved for fractured bones, subcutaneous bruises, and diabetes mellitus. In the diseases of the urinary organs a slight grade of fatty degeneration of the kidneys may explain the condition, which occurs in nephritis, various infections, intoxications, anæmias, and cachexias. The fat may also arise from the fatty degeneration of epithelial cells, leucocytes, casts, and fragments of tumors; in such cases the most of the fat remains in the cells, or collects in droplets which float on the surface.

**Organized Sediments. Mucous Sediment.**—The “nubecula” is a very faint cloud of mucous strands appearing soon after the urine cools, which first collects at the top, then sinks to the bottom of the glass. It is mucus from the epithelial cells of the urinary passages. These strands enclose a few “mucous corpuscles,” mononuclear or polymorphonuclear leucocytes often some amœboid, and some crystals. When much mucus is present in the urine it may form a translucent or cloudy coagulum-like sediment which is more clearly seen after the addition of acetic acid. This is the product of desquamatory catarrh of the mucosa.

**Epithelial Cells.**—It is normal for a few cells to be present in the nubecula, since the mucosa of the urinary passages is an epithelial surface, hence is always desquamating somewhat. These cells are increased in inflammatory and destructive lesions, in which case cells

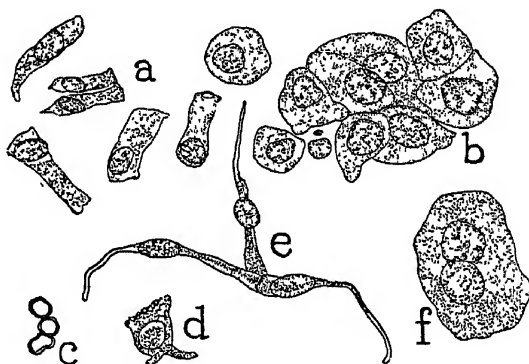


FIG. 45.—a, e, d, cells from male urethra; b, f, cells from transitional epithelium; c, shadows of red blood-cells.  $\times 400$ .

from the lower layers, which are normally never present, may also appear.

**RENAL EPITHELIAL CELLS.**—The cells from the kidney (see Fig. 47, e) are round or cubical, a little larger than leucocytes (12 to 25 microns) from which they are distinguished by their size and nucleus. The latter is large and vesicular, especially well seen if stained. The protoplasm is nearly always fatty, either finely so, or so very fatty that it may resemble a colostrum corpuscle (c, h). These cells sometimes show definite myelin degeneration and free myelin globules similar to those in the sputum (see Fig. 47, d).

These are very rare in normal urine, occur in any form of nephritis, but especially the acute parenchymatous, singly, in clumps, or attached to casts, and in renal infection in masses of pus-cells (see Fig. 47).

**EPITHELIAL CELLS FROM THE URINARY PASSAGES** (see Fig. 46, b, c, d, and Fig. 45, b, f).—These cells may be large and irregular,

round or polygonal; they are flat, with clear protoplasm and usually with a small, very distinct central nucleus. Their edges are sometimes very refractive, thin, and horny. These are the typical pavement cells from the superficial layers of transitional epithelium. In cases in which they are largely increased, as, for instance, the result of too strong irrigating fluids, they may occur in large sheets. Dawson<sup>153</sup> found that these cells varied in size and shape, some being irregular, large, and polygonal, some smaller and hexagonal, the larger often having a peripheral non-granular zone. The nucleus was round or oval, sharply defined and central, and many were budding.

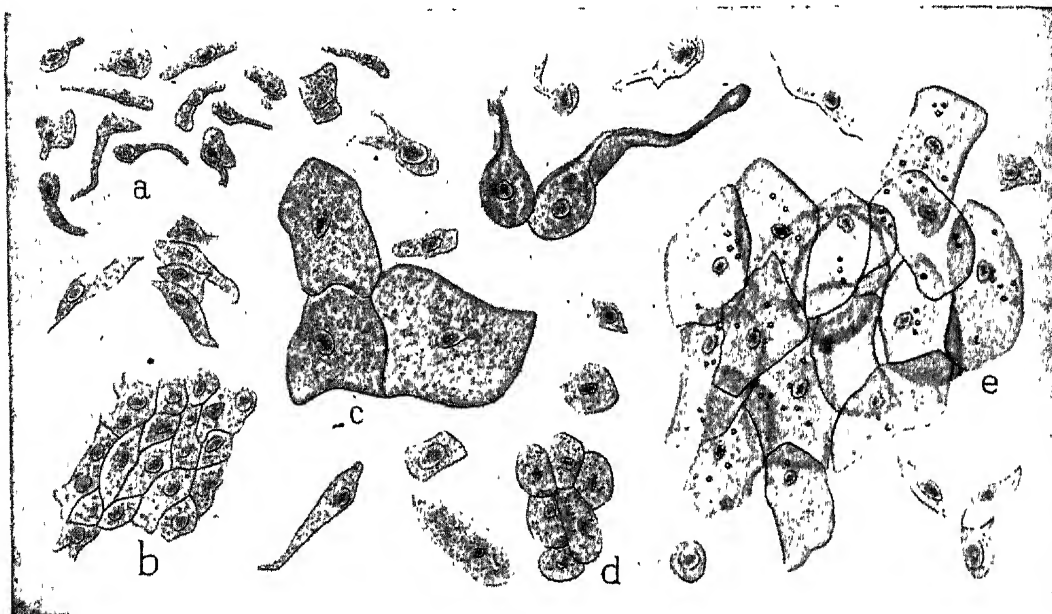


FIG. 46.—Various forms of epithelium cells in the urine. a, "tailed" cells; b, d, small polygonal; c, large surface cells; to the right of d is a small round cell of uncertain origin; e, squamous cells from vagina. All of these cells except e were obtained by ureteral catheterization, hence from the pelvis of the kidney or scraped from the mucosa of the ureter. The latter is especially true of b, c, d, and neighboring cells, which are the forms one gets from normal cases. a were from cases of pyelitis.  $\times 400$

Among these cells were large giant-cells with fifteen nuclei. In no cells did he see the cupping of the under surface supposed to be present.

The flat polygonal SQUAMOUS EPITHELIAL CELLS (see Fig. 46, e) from the prepuce, end of the ureter, vagina, and fossa navicularis, cannot always be distinguished from the superficial cells from the bladder, although usually the stratified grouping of those from the vagina makes diagnosis easy.

The CYLINDRICAL CELLS (see Fig. 45, a, e, d) of the urethra are longer, bluntly pointed, and smaller than the above, and occur in pairs or clusters.

There are found smaller polygonal or elliptical cells from the other layers of the mucosa of the bladder, ureter, and pelvis, which consist of a very granular protoplasm and a large nucleus (Fig. 46, a, b, d). Other cells are more oval, often irregularly conical, with one or two branches. Their nucleus is very distinct, their cell body long and thread-like. In addition are small round cells, with round nucleus like mononuclear leucocytes (see Figs. 46, 47), which, indeed, they may be, or cells from the deep layers of epithelium. We have seen good numbers of these round and tailed cells in the urine obtained by ureteral catheterization from normal kidney (always with some blood).

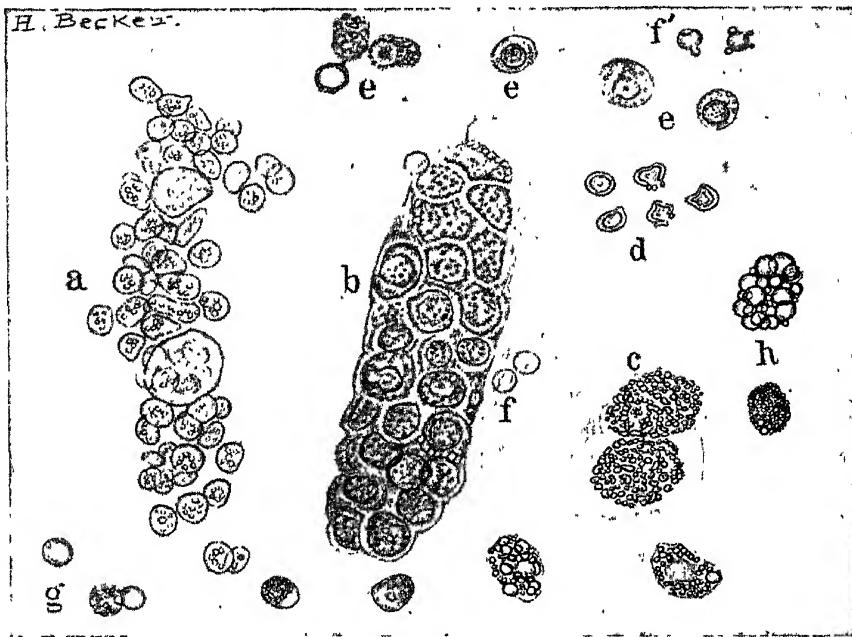


FIG. 47.—a, pseudo pus-cast; b, epithelial cast showing protoplasmic bridges between cells; c, two very granular (myelin?) renal cells; d, myelin globules; e, renal epithelial cells; f, crenated red blood-cells; g, pus-cells; h, very fatty renal epithelial cells.  $\times 400$ .

These come from the middle or deeper layers of the mucosa, anywhere from the pelvis of the kidney to the bladder, singly or in clusters.

Some suppose that they can recognize the cells from the various parts of the urinary passages. Others, and this is our opinion, believe that this is usually impossible. Sahli considers that the predominance of tailed cells over flat cells indicates a pyelitis. We have seen such cases, but in a recent case of intense pyelitis, the urine obtained at autopsy from the pelvis of the kidney, the point failed. A former idea was that all tailed cells came from the pelvis of the kidney. The smaller polygonal cells from the ureter (Fig. 46, b, d) in groups are suggestive. These are the cells scraped off by the ureteral catheters.

In a recent case of streptococcus pyelitis the urine from the pelvis of the kidney showed great numbers of small round or polygonal and tailed epithelial cells in groups of considerable size, scores in each field (of 400 magnification), and of the large polygonal cells three to four in each field. Pus-cells were in great numbers; little mucus was seen.

**Casts.**—These have been divided into cellular, granular, and amorphous; the latter show no structure, but are homogeneous or with a faint striation. All combinations and transitions of these casts, and casts with various elements fastened to them, occur.

**EPITHELIAL CASTS** (Figs. 47 and 50).—These are made up of renal epithelial cells; in some cases aggregations of desquamated cells are massed together; others are certainly desquamated portions of the tubules, presenting a lumen, and having the intercellular protoplasmic bridges visible (Fig. 47). In one kidney which we have had opportunity to study, in sections of the cortex the invaginated



FIG. 48.—Coarsely and finely granular casts.  $\times 400$ .



FIG. 49.—Waxy casts.  $\times 400$ .

tubules of epithelium could be easily seen, which breaking off would give casts. Such perfect fragments are called "epithelial tubes." The cells may be well preserved, or present a marked fatty or granular degeneration. The nuclei are round and vesicular; for the recognition of the cast it is necessary to determine this point. All transitions between these and coarsely granular and fatty casts are seen.

**GRANULAR CASTS** (Fig. 48).—The granules may be coarse or fine. The former give to the cast a yellowish-white color. All transitions between the epithelial or leucocyte and coarsely granular casts may be found. The granules are soluble in acetic acid. To these casts may be attached epithelial cells, leucocytes, or red blood-cells. The coarsely granular casts probably represent the granular disintegration



of epithelial casts, and are formed either from epithelial tubes or from masses of cells which have previously undergone such disintegration; the outline of cells can sometimes be seen, and fat globules are commonly also present. This term often includes the hæmoglobin casts which are of a brownish red color. But another group of finely granular casts is somewhat different, and transitions between these and the opaque finely granular are not common; that is, not in the same case. These casts are covered by very fine granules, are less opaque than the coarsely granular, and fat droplets are not commonly present. When the cast is only partly finely granular the rest is hyaline; in the case of the coarsely granular it is waxy.

**FATTY CASTS** (Fig. 50).—These striking objects are masses of fatty globules, often preserving the outlines of the original epithelial cells. They are yellowish or even blackish in appearance, soluble in

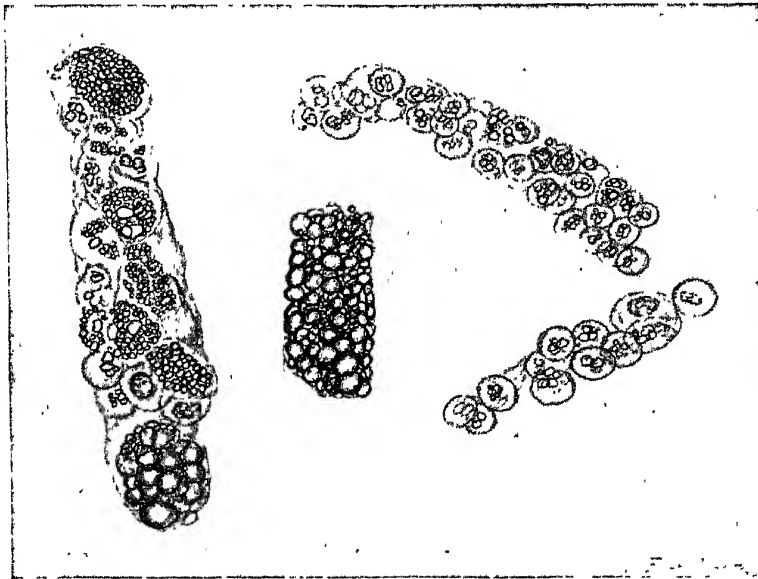


FIG. 50.—To the left an epithelial cast with very fatty cells; in the centre a fatty cast; to the right two leucocyte casts.  $\times 400$ .

ether; fatty acid crystals may project from them. If any cell outline can be made out, the cast usually is called epithelial.

**WAXY CASTS** (Fig. 49).—These casts are very refractive, sharply contoured, often of a white or yellowish color, homogeneous, and show a great tendency to split transversely, hence sometimes are in very small pieces. This appearance of great brittleness is quite characteristic. They may have any cellular elements attached. They are probably a further modification of the granular detritus of epithelial cells. Their appearance is that of wax. They are broader than the hyaline. Some give the amyloid reaction, others do not. They

are not characteristic of the amyloid degeneration, as was formerly supposed, and yet in a recent case of amyloid disease practically every cast was a waxy cast. In general there are two very distinct forms of waxy casts,—the yellow and the blue. The former were often called fibrin casts; they resemble beeswax, the latter paraffin. These occur in any nephritis with granular casts, especially when the urine is diminished, or just before death.

In the urine obtained just before death one may see the most beautiful waxy casts. In one such case recently there were many granular and waxy, no hyaline. The casts were enormous, many granular being 0.136 mm., and waxy 0.102 mm. in diameter. The latter looked as if cut out of paraffin. But this is not always the case. In another specimen only hyaline casts were present, no waxy. But these casts were not typical hyalines, yet they were not at all refractive. In other cases all forms may be found. The great difference between hyaline and waxy casts is their refractivity, and it is hard to believe that they are not directly related.

Between this group and the next is a very large group of casts, the commonest form in some cases of nephritis, which have not the physical properties of wax, yet are more distinct and solid-looking than the hyaline, which name they bear. It is important to recognize that some of these have deposited on them fine granules from the urine, giving them the appearance of the finely granular casts. Their chief difference from waxy casts is that they are not so solid-looking and do not give the same color tests.

**HYALINE CASTS, COLLOID OR GLASSY CASTS** (see Fig. 51).—These are pale, very little refractive, watery in appearance, and difficult to see unless the light is almost shut off, or unless crystals or cells are attached to them. It is advised to stain them with Lugol's, giving them a yellow color, or aniline-violet, giving blue. They give the micro-chemical tests for albumin. They may have the same cells attached as the above mentioned casts. Their outline is very regular. These casts, which occur in circulatory disturbances where there is no question of inflammation, are so different in appearance from the hyaline casts just mentioned that they deserve a separate name. They are soluble in acetic acid.

**BLOOD-CASTS** (see Fig. 52).—Blood-casts are coagula of red blood-cells which have formed within the tubules. The term is also applied to any of the above casts with red blood-cells attached. Some of the blood-cells are so pale that it is hard to recognize them.

**HÆMOGLOBIN**.—Casts of hæmoglobin are seen in hæmoglobinuria, the hæmoglobin occurring in amorphous masses. Some seem like other casts impregnated with hæmoglobin.

**PUS-CASTS** (see Fig. 50) are formed in similar way as the blood-casts. The nuclei must be seen and their polymorphous nature certain, to be sure it is not an epithelial cast. This may be done by adding

acetic acid. Another point to differentiate from epithelial casts is the spherical shape of the pus-cells. These casts are rare, yet often other casts are seen with leucocytes attached which go under the same name.

CYLINDROIDS (see Fig. 53).—It is common and right to divide these into two groups. The first is of the so-called mucous threads, which are flat ribbons of mucus which no one would confuse with hyaline casts. Their length is considerable, several fields in fact; they vary in diameter, on the whole are narrow, and clearly show their ribbon-like nature. Such threads make up the nubecula. In addition to these and differing much from them in appearance are seen elements which look much like hyaline casts for the most of their

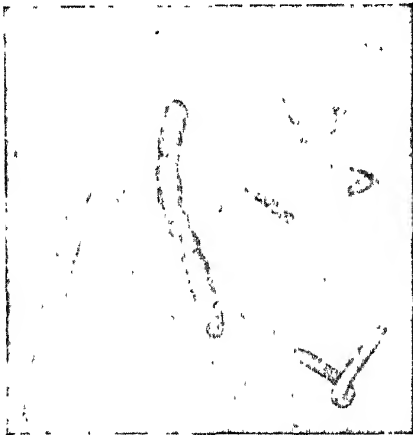


FIG. 51.—Hyaline casts of urine.  $\times 400$ .

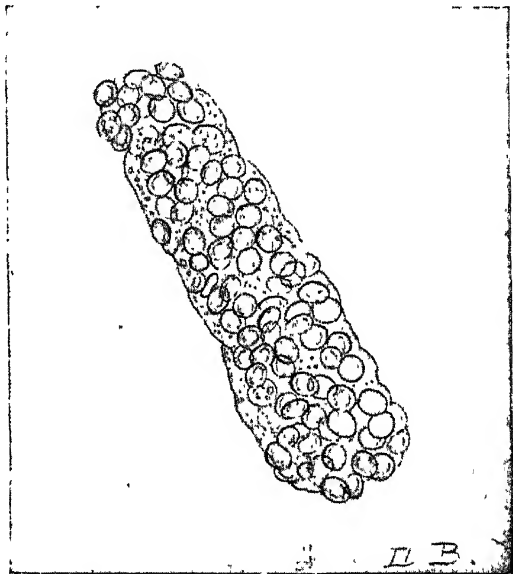


FIG. 52.—Blood-cast.  $\times 400$ .

length, but at one end run off into a longer or shorter thread. Those particular on this point exclude from the list of casts anything which has one end at all tapering and thread-like. These casts appear to be circular on cross-section. They have not the fibrillar nature of mucous threads. They occur where casts would be expected, practically always with true casts, and have the same significance as they. Of one thing we are quite certain, that for the most of their length they are typical hyaline casts, and when, as may occur in the centrifuge, the thread-end is broken off, the other fragment could not be distinguished from a cast. The cylindroids may be covered by urates, and hence have the appearance of granular casts. Chemically these are like casts. The point is of considerable importance, for if mucous threads they certainly arise from the mucous surface, while if casts they should arise in the renal parenchyma. They were first described as of

some significance as casts. It is perhaps safest to observe the old rule and exclude all from the list of casts which have a definite tail at one end. The true mucous threads are insoluble in acetic acid. Their origin is the bladder chiefly. One seldom sees them in urine catheterized from the pelvis of the kidney.

**COMBINED CASTS.**—A cast may be waxy or hyaline at one end, granular at the other, or may have cellular elements attached. They take their name from the latter.

**BACTERIAL CASTS.**—Masses of bacteria in the shape of a cast occur in purulent infectious pyelonephritis and in pyæmic kidneys, but a cast also may become permeated by bacteria, either in the body or after voiding, in a remarkably short time.

**URATE CASTS.**—In uric acid infarcts of the kidney of the newborn masses of sodium urate may be found in the urine.

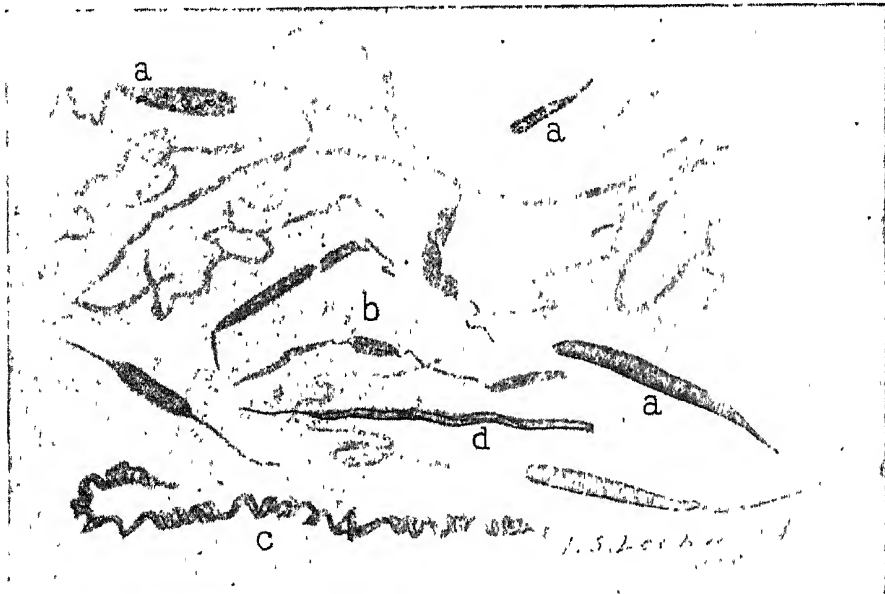


FIG. 53.—a, cylindroids, *i.e.*, bodies much resembling hyaline casts; b, mucous threads; c, a spiral structure of material resembling hyaline casts or mucous threads; d, a vegetable thread.  $\times 400$ .

**PSEUDO-CASTS** of urates are commonly seen. It is not at all unusual for a urate sediment to assume this form. All casts in a concentrated urine may become incrustated with urates, and hence be of a dark homogeneous granular nature. True granular casts are not homogeneous, are coarser and more refractive than these pseudo-casts, which also have uneven edges and disappear on warming. Scratches in the glass are sometimes confusing (Fig. 54). In some cases of pyuria (cystitis, *e. g.*) the pseudo-casts are very perfect (Fig. 47).

The *length* and the *breadth* of casts vary. Their size may be from very small fragments to 1 mm. in length or longer. These very long casts are almost always hyaline, and are not mucous threads. Their diameter is narrow or broad. From

the size of the casts no conclusions can be drawn of their source, so much does the size of the tubules vary in various conditions. It was formerly supposed that the beautiful corkscrew forms so often seen came from the convoluted tubules, but this is improbable, since any corkscrew shape would certainly be effaced during their passage through the straight tubules. Some are spiral all the length, others only at one end. This, says Senator, merely shows them composed of a tough elastic material which has been squeezed through a narrow canal or through a narrow orifice. The end of the cast is seldom split or forked. Some casts are almost on the limit of vision, but vegetable fibres must be excluded.

The *origin* of epithelial casts, especially those with a lumen, is not disputed; also that of blood and pus casts, which are conglomerates of cells in tubules. The coarser granular casts quite certainly are formed from epithelial casts by a granular degeneration of the cells, and all transitions from the coarsely granular casts to the waxy may be found. This transition may also occur in the leucocyte casts, but is less true of the blood-casts. Such transitions are followed best by the study of sections of the kidney. The origin of the hyaline casts, however, has long been in dispute, some claiming that they were a coagulated exudate from the

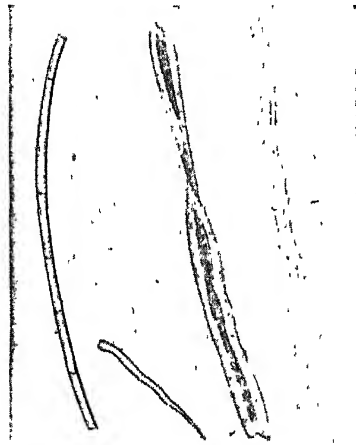


FIG. 54.—Pseudo-casts. From left to right, a linen thread, a vegetable spine, a cotton thread, and a scratch on the glass slide.

blood into the tubules, others a product of the secretion of the epithelial cells. Hyaline globules are present in these cells, the confluence of which in the tubules could easily explain casts; that is, the very slightly injured epithelial cell, still functioning, may by an abnormal secretion of coagulable material form these moulds of the tubules. This latter is the generally accepted idea. Also these epithelial cells could die, in part or wholly, undergo hyaline degeneration, and this substance be moulded into a cast. This also may be seen in sections. They are certainly not coagulated fibrin, since they arise where there is no suspicion of inflammation, that is, in a practically normal kidney, as in the albuminuria of the newborn; they do not give the Weigert's fibrin stain; where a coagulable albumin is present, as in the case of chyluria, these casts are absent; also there is no relation between albumin and casts in amount, and one may be present without the other. There is therefore no evidence for the albumin of the blood as their source. It is possible that a difference in origin explains the two forms of hyaline casts. Since casts which remain long in the tubules in cases where hyalines are the rule are waxy, the transition from hyaline to waxy must be considered.

The origin of hæmoglobin casts is interesting, since hæmoglobin is soluble in the urine. It may be, however, that they are hyaline or waxy casts impregnated with hæmoglobin.

The chemistry of casts has been but little studied. Probably none are composed of fibrin. Whether any consist of amyloid or not is disputed. Very few give a typical amyloid reaction, the majority of those which look waxy taking a merely brownish color with Lugol's solution, and a reddish color with gentian-violet. Most genuine casts are soluble in acetic acid, which is a valuable aid in distinguishing between them and mucous cylindroids.

**Diagnostic Importance of Casts.**—It is claimed that casts are sometimes found in the urine of persons whose kidneys are in normal condition. This is not generally admitted. It is the opinion of most writers that casts are always evidence of an abnormal condition of the renal epithelium, a disturbance which may be slight and transitory and of little significance, as a temporary condition of malnutrition, a mild irritation, congestion, pressure, etc.; or permanent and serious, as in nephritis. It is also true that from the number and character of the casts present in the urine one cannot judge as to the severity of the cause of the cylindruria. Indeed, it would seem that, the more normal the condition of the cells prior to the renal disturbance, the more brilliant will be the display of casts. Certainly the most seriously injured (small, contracted) kidneys may for fairly long periods excrete urine containing few or no casts.

The general statement may be made that casts appear under the same circumstances under which albumin does or might appear, and that both albumin and casts have the same general significance, though it would seem that of the two the casts are a more sensitive index of renal disturbance than is albumin.

But, while albuminuria and cylindruria are usually associated, yet either may exist without the other. Casts have been reported absent when albumin was present in some cases of chronic nephritis (especially the arteriosclerotic type), in some cases of jaundice, and in some of febrile albuminuria. To prove casts absent, however, is no easy matter, since in some urines they disintegrate very rapidly. The urine should be examined for them while it is very fresh. It should be centrifugalized, and the sediment at once carefully studied. The rapid disappearance of casts from some urines has been ascribed to the presence of a ferment which some claim is pepsin, others, a bacterial enzyme.

On the other hand, it is not at all rare to find casts in urines which are practically free from albumin. The sources of that irritation of the kidneys which causes a pure cylindruria may be some food, as asparagus, radishes, coffee, mustard; a drug, as alcohol, salicylic acid, mercury, arsenic, camphor; an injection of tuberculin; also many other irritants. Pure cylindruria is sometimes seen in cases of heart disease, cancer, jaundice, and constipation; in acute

infectious diseases, as scarlet fever, typhoid fever, erysipelas, tuberculosis; in nephritis, especially during convalescence, even in uræmia. In fact, in any case in which albuminuria would be expected, cylindruria may occur. It is of interest that among the athletes studied with reference to "physiological albuminuria" were several in whose urine there were casts, but no albumin.

In a recent case of brain tumor we found many epithelial and waxy casts and many cylindroids, but no albumin.

In these instances the cylindruria is doubtless caused by an irritation of the renal epithelium which is not sufficiently strong to induce albuminuria; there is little doubt that in other cases both casts and albumin were for a time present, but the latter disappeared first, while the cylindruria continued. We believe that the casts are apt to disappear before the albumin in those cases in which the renal lesion is chiefly degenerative, as in patients with arteriosclerosis; the albumin first, in those cases in which the lesion is an inflammation, as it is in parenchymatous nephritis.\* Without much doubt our failure to find more cases of pure cylindruria has been due to our lack of interest in examining carefully the very fresh urine of patients whose urine contains no albumin.

The association of casts and "nucleo-albumin" is often noted in such cases as are marked by the absence of serum albumin.

In any given case of nephritis there is a rough parallelism between the number of casts and the amount of albumin in the urine. The different forms of nephritis, however, differ much as regard the cylindruria present. Casts are most abundant in acute and subacute parenchymatous nephritis, and fewest in interstitial nephritis, amyloid disease, and chronic passive congestion. In the diagnosis of the grade of a case of renal trouble the number and variety of casts are not as important as the specific gravity of the urine, its chemical analysis, and especially the physical examination of the patient (Osler). There are no casts which are pathognomonic of nephritis; any or all varieties may appear in the urine of patients with nephritis and also of patients who have no Bright's disease. But nephritis is practically the only condition in which cylindruria is long-continued; in other cases it lasts but a few hours or days.

"Showers" of casts are very interesting finds in the urine. Suddenly and for a short time, usually a part of one day, the urine contains a number of casts which is greatly in excess of that of the preceding or of the succeeding day. This may occur in any form of nephritis.

Pure cylindruria occurs in some cases of chronic nephritis and of waxy kidney. Stewart tried to separate a group of severe nephritis

\*Emerson, Johns Hopkins Hospital Bull.; Vincent, N. Y. Med. Jour., April 13, 1907.

cases which presents this feature, but the anatomical evidence for such a group is still lacking.

In cylindruria not due to nephritis the casts are few, and those found are hyaline, as a rule.

The casts of chronic passive congestion are scanty if any, and nearly all are hyaline. Yet all gradations occur between this condition and true nephritis.

It was formerly supposed that the epithelial and the hyaline casts meant an acute process, the granular and the waxy a chronic process; but in any form of nephritis all kinds of casts may occur; in amyloid disease even there is in the urine nothing characteristic. Sahli suggests that the granular and the waxy casts become such from lying a long time in the tubules, which occurs in acute nephritis or acute exacerbations of a chronic case, in which the secretion of urine is much suppressed. We have noticed this point also in other oligurias following, for instance, decapsulation of the kidneys. For the first few days, as the urine begins to increase in amount, a very large number of waxy casts were found.

Following renal disturbance of almost any kind casts may appear. Brown<sup>155</sup> has reported some interesting cases in which operations upon the kidney, nephropexy or exploratory nephrotomy, the urine previously being normal, were, the first day after the operation, followed by an output of casts in enormous numbers. These were hyaline, granular, blood, and epithelial. The field may be really crowded with them. Considerable albumin may be present. They diminish rapidly in amount, and in a few days (from two to six) have entirely disappeared. During this time there have been no symptoms of nephritis, no oedema, and the amount of urine has been normal. The most striking urinary feature was the disproportion between the amount of albumin and the number of casts, this being greatly in favor of the latter. There were no later symptoms.

Epithelial and leucocyte casts are not nearly so rare as is imagined, and may occur in even non-inflammatory transitory cylindrurias.

Casts are considered important as a prodromal symptom of diabetic coma (Külz). Before the coma begins casts appear in immense numbers, and even may form a macroscopic sediment. These casts are of characteristic appearance,—short, broad, of delicate outline, pale, the most of them granular and hyaline, and with few other formed elements.<sup>156</sup>

**Staining Casts.**—This is unsatisfactory, because the stain precipitates in the urine, or the albumin of the urine may itself take the stain. The specimens cannot

<sup>155</sup> Johns Hopkins Hosp. Bull., May, 1900.

<sup>156</sup> See Domansky and Reimann, *Zeitschr. f. Heilk.*, 1901, and Herrick, *Am. Jour. Med. Sci.*, vol. cxx, 1900.



be dried for this reason. The casts should be washed by one or two sedimentations with 0.6 per cent. sodium chloride solution to rid of all soluble matter and albumin. In the next centrifugalization 1 per cent. methylene blue may be added. To hasten centrifugalization a little alcohol should be added, not much, nor allowed to remain in contact with the sediment for a long time, else a coagulum will result.

To preserve the casts and also to stain them they should be washed in the above normal salt solution, and lastly in a 1 per cent. osmic acid solution or in 1 to 10 per cent. formalin, or in a 5 per cent.  $\text{HgCl}_2$  solution for five minutes. In the latter case they are then washed with water and preserved in from 2 to 10 per cent. (or 1 to 2 per cent.) formalin. If no red blood-cells are present, the mercuric chloride may be omitted. This salt disturbs microchemical tests. In case formalin is used the casts should be well washed once or the spherical crystalline masses of diformaldehydurea will form. Gumprecht adds that it is not really necessary to wash the casts if they are well centrifugalized and the supernatant fluid completely decanted. A good staining method for fat and cell nuclei was described by Cohn.<sup>157</sup> The specimen, well washed by centrifugalization in normal salt solution, is air-dried on the cover-glass and hardened by immersing the glass in 10 per cent. formalin for ten minutes. It is then washed rapidly but gently with  $\text{H}_2\text{O}$  and then immersed for ten minutes in a concentrated Sudan III. solution in 70 per cent. alcohol. It is then washed in 70 per cent. alcohol for one to two minutes and then stained briefly in the hæmatoxylin (Ehrlich's). The specimens are mounted in glycerin.

Kozłowski<sup>158</sup> recommended Farrant's mounting fluid, consisting of equal parts of water, glycerin, and saturated aqueous solution of arsenic acid (saturated by weeks of standing); to this gum arabic one-half volume is added, and the whole allowed to stand (about three weeks) till all is dissolved. It is then filtered if necessary.

In the centrifuge tube are mixed 1 cm. of 1 per cent. eosin or methyl violet with the urine, then centrifugalized and washed by centrifugalization till all the urine is removed. One drop of sediment is then mounted on the slide with one drop of the above fixing fluid.

Bohland advises to wash with salt solution; then Müller's fluid is added, and they kept in this for two weeks, changing the fluid two to three times. The Müller's fluid is then decanted and the sediment washed in absolute alcohol until this is colorless.

**Testicular Casts.**—In "spermatorrhœa" casts have been described which "can hardly be distinguished from renal casts except that the urine is otherwise normal. They are all in the first glass of the two-glass test, and the presence of spermatozoa will indicate their origin. They are supposed to arise in the testicle." We have inquired of those with a very wide experience in the examination of prostatic secretions, and they say they have never seen such structures. *Spermatozoa* may often be found, active at first, and with all of the elements of unripe semen. They soon go to pieces. Such occur not only after coitus and pollution, but after epileptic and other convulsive seizures.

**Tripperfäden.**—These occur in a late stage of acute gonorrhœa and in chronic gleet when the secretion becomes very mucous and collects in the longitudinal furrows of the mucosa. They may be from a few millimetres to one centimetre long and yellow or white in color,

<sup>157</sup> Zeitschr. f. klin. Med., 1899, Bd. 38.

<sup>158</sup> Virchow's Arch., 1902, vol. clxxix. p. 161.

consisting of a mucous ground substance in which are embedded pus and epithelial cells.

**Tissue fragments, portions of carcinoma,** have been found; also masses of caseous matter in cases of tuberculous ulceration. The elastic tissue may be demonstrated, and in the case of cancer the spindle cells, which may enclose hæmatoidin crystals and red blood-corpuscles. To demonstrate elastic tissue the urine should be centrifugalized, acid added to dissolve the phosphates, the supernatant fluid decanted, and the sediment then warmed with an equal amount of 10 per cent. KOH. This destroys all but the elastic tissue. It is then sedimented again and examined microscopically.

In papillomatous cancers and growths of the bladder fragments large enough to be cut in sections may be found. They have been found in the urine especially of cases of carcinomata of the bladder, very rarely of the kidney. From some of these a diagnosis could be made. Fragments of sarcomata in the urine are almost unknown, but structureless masses have been found, as by Rothschild,<sup>159</sup> from a giant-cell sarcoma of the kidney. This large mass was 5.2 cm. long and 0.5 cm. wide, structureless, glassy, transparent, and quite firm.

Other gross masses are mucous casts (see page 227), and fibrin masses in chyluria, hæmaturia, and from inflammatory conditions, especially tuberculosis.

**Pus-Cells.**—These occur, a few perhaps, in normal urine, but many in any inflammation of the urinary passages, of the kidney, or in case an abscess ruptures into the urinary tract. Their numbers vary enormously. As a rule, if from the cortex of the kidney, they are few in number, if from the passages, many.

Hottinger found in a case of cystitis 150,000 leucocytes per cubic centimetre, that is, a daily output of about one one-hundredth the number of leucocytes normally at one time in the circulating blood.

Their origin is better indicated by other constituents, as, for instance the character of the epithelial cells or the presence of casts. In case a large amount of pus appears suddenly, the probable source is ruptured abscess. The pus-cells in gonorrhœa may be mixed with mucus, form threads, the so-called Tripperfäden which probably are formed in the folds of the mucosa and are washed out by the stream of urine. These Tripperfäden will settle to the bottom of the glass. They should be searched for only in fresh specimens by agitating the urine, when they arise as long threads, since if allowed to settle long they will coalesce. They have a considerable diagnostic value.

In all cases of women pus from the vagina should be excluded.

In alkaline urine the pus-cells will swell and the mass become slimy and gelatinous. The pus sediment is, as a rule, slimy, since it

<sup>159</sup> Deutsches med. Wochenschr., 1901, No. 50.

contains so much mucus. Albumin is always present. Microscopically in acid urine the cells may be cloudy and shrunken, and acetic acid be necessary in order that the nucleus may be seen at all. In an alkaline urine they will swell and become glassy, but even here it is not easy to see the nucleus. In a weakly alkaline, amphoteric, or faintly acid urine they may be well preserved for a long time, and even show active amoeboid motion. Their diameter is from 7 to 12 microns. Their nucleus is small, usually polymorphous, never vesicular. This will exclude renal epithelium, which it is often hard to do except in stained specimens. (Senator considered that many of the pus-cells in Bright's disease were mononuclears.)

We have had opportunity recently to examine two cases in which the leucocytes were so drawn out that they resembled spindle epithelial cells. This may have been due to long centrifugalization in one case. This was true of practically all the leucocytes and on two preparations.

It is often important to decide whether there is more albumin than the pus serum would explain; that is, if true albuminuria is also present. In case casts and renal epithelium are found a cortical origin for some at least of the albumin may be assumed.

**Posner's Method.**—The albumin is estimated carefully. The urine is well shaken (a twenty-four hours' specimen) and the leucocytes counted with the ordinary blood-counter. For each 100,000 leucocytes per 2 cc. of urine, one may expect 0.1 per cent. albumin (Goldberg, 2 p. M.). The urine must be diluted with 1 to 3 per cent. NaCl solution if over 3000 per cubic millimetre are present.

The same author has given an easier method which is of some value. The urine is poured into a flat-bottomed beaker which is placed over a sheet of ordinary printed paper. The well-shaken urine is then poured in until the type cannot be read. Normally one can read through a layer of urine 8 cm. deep; if the type cannot be read when the layer is from 0.5 to 1 cm. it indicates 40,000 leucocytes per cubic centimetre; if 6 cm., 1000 leucocytes per cubic centimetre. The benefit of treatment may be followed by this method. A former idea, that the filtrate of a urine without true albuminuria is albumin-free, is hardly true, although the albumin in the filtrate is of very small amount.

**Donné's Pus Test.**—The supernatant fluid is poured off, and to the sediment a small piece or strong solution of KOH or NaOH is added. If the sediment is pus it will be transformed to a viscid gelatinous mass which sticks to the glass.

The pus-cells will take a mahogany-brown color with Lugol's.

**Red Blood-Corpuscles apart from True Hæmaturia.**—These are present in the urine in acute inflammations of the kidney, tumors of the urinary passages or kidney, in cases of trauma, stone, chronic pas-

sive congestion, the hemorrhagic diathesis, after severe exercise as long foot-races (Barach) and in many trivial conditions in which they would not be expected. Some of the cells are intact or they may appear as shadows.

In concentrated urines they are crenated; in dilute, swollen or laky; in acid urines, intact; in alkaline, destroyed, forming masses of yellowish-brown granules.

It is important to decide if these cells come from the cortex of the kidney or not. A cortical origin may be assumed if many red blood-cells are sticking to casts or if true blood-casts are present. An amount of blood sufficient for clot formation usually has its source below the cortex, but sometimes in nephritis enough blood may escape from the cortex to form large clots, the shape of which will sometimes indicate the source, it being a cast of the pelvis or of the ureter.

Gumprecht claimed that if many of the reds were found fragmented, that is, present as clumps of granules, the source in the cortex is to be assumed, since here alone the urea solution is strong enough to fragment the reds (8 per cent.). Goldberg thinks that these cells can become fragmented in a distended infected bladder.

**Renal and Bladder Stones.**—By renal stones are meant all from the pelvis of the kidney and the ureter. They vary in size from a grain of sand to those which fill the whole pelvis of the kidney. These large ones with the branches may be hollow, furnishing thus a passage for the urine (in one case it weighed 1088 grains). The bladder concretions are single or multiple, and vary greatly in size.

**Uric Acid Concretions.**—These are very common, the most common renal stone. The size of those found in the bladder varies from that of a pea to that of a goose-egg. They are always colored, most commonly a grayish yellow, yellowish-brown, or a pale reddish-brown. The surface is sometimes smooth and polished, sometimes rough and nodular. They are very hard, fracture easily, and on cross-section they show a concentric arrangement and crystalline structure, the layers of which may be separated being of different colors. These layers may be alternately of uric acid and some other salt, as, for instance,  $\text{CaOx}$ . They burn without residue if pure, they give the murexid test, on the addition of  $\text{NaOH}$  they liberate but little ammonia, are soluble in alkali, and this solution plus acetic acid gives crystals for the murexid test.

**Ammonium urate stones** are primary in the new-born, and occur rarely in adults. As secondary deposits they occur more commonly. These stones are almost as soft as wax, when dry are clayey and easily powdered. They give the murexid test and with  $\text{NaOH}$  liberate much ammonia.

**Calcium oxalate stones** are, next to uric acid stones, the most common,

and yet seem the most numerous, since they cause severest symptoms. They are either smooth and small or very large with a rough nodular or ragged surface, of a dark gray to blackish color. They cause hemorrhage easily, and hence are often stained dark brown with blood-pigment. They are the hardest and heaviest of stones. They are soluble in HCl without gas formation, but not in acetic acid. After moderate heating, however, the powder is soluble in acetic acid with gas evolution. After strongly heating, the powder reacts alkaline because of the  $\text{Ca}(\text{OH})_2$  formed. They contain almost always some uric acid, xanthin, or calcium carbonate, and hence have a concentric layer arrangement.

**Phosphate Stones.**—As renal stones they are rare and small, yet often occur as ingredients of other stones. They usually consist of a mixture of normal phosphates of the alkaline earths and triple phosphate. In the bladder they may be very large. They occur especially as secondary concretions, and contain ammonium urate,  $\text{CaOx}$ , and carbonates. They often form around a foreign body. Their color varies,—sometimes white or pale yellow, or purplish. They are soft, light in weight, the surface always rough. Concretions of triple phosphate alone are rare. They are small with a granular surface, upon which are often red crystals. Stones of the ACID CALCIUM PHOSPHATE are rare. They are white and of a beautiful crystalline structure. These phosphate stones do not burn, the powder is soluble in acetic acid without gas formation, and the solution gives the reaction of phosphoric acid and of alkaline earths. They usually contain a great many organisms. The TRIPLE PHOSPHATE STONES liberate much ammonia on the addition of NaOH.

**Calcium carbonate stones** are rare in man, are chalky white, soluble in acid with gas formation.

**Cystin stones** are rare. Their size varies, and may reach that of a hen's egg; as renal concretions they are seldom larger than a small pea. The life of some of the cases of cystinuria is wretched because of the rapid formation of stones large enough for operation. They are light in weight, smooth, soft and waxy in consistency, hence may usually be crushed. They have a smooth or ragged surface, are white or pale yellow, and crystalline on cross-section. They are rather wax-like, burn readily and perfectly on a platinum-foil with a bluish flame, are soluble in ammonia, recrystallized by acetic acid, and give the other cystin reactions.

**Xanthin stones** are very rare and occur especially in children. This is also a primary formation. They vary in size from a pea to that of a hen's egg. They are pale white, yellowish-brown, rather hard, amorphous on cross-section, and on rubbing appear like wax. They burn without residue on the platinum-foil, and give the xanthin test.

**Fatty concretions** have only a few times been observed. They contain free fatty acid, neutral fat, and much cholesterin. In some cases these have been found to be of the fat used in passing bougies.

**Indigo.**—Three such stones are on record, and yet this may be the nucleus of various other stones. They have a blue or bluish-gray surface.

**Albumin.**—One such calculus is on record.

# WHEN HEATED ON THE PLATINUM-FOIL THE POWDER

(HOFMEISTER'S TABLE.)

Does not burn		Burns					
The powder + HCl		With flame		Without flame			
Does not effervesce		The flame is yellow, continuous, odor of burning feathers. Insoluble in alcohol and ether; soluble in hot KOH, and reprecipitated white by acetic acid, with H <sub>2</sub> S formation.	A yellow, clear continuous flame, odor of resin or shellac; the powder soluble in alcohol and ether.	Flame pale blue, burns for a short time with a characteristic sharp odor. The powder is soluble in NH <sub>4</sub> OH and on evaporating hexagons are precipitated.	Does not give the murexid test. The powder is soluble in HNO <sub>3</sub> without gas effervescence, and this dried residue becomes orange when KOH added, then red on warming.		
The powder moderately burned + HCl						The powder gives the murexid test.	
Does not effervesce							The native powder on the addition of a little KOH in the cold
The native powder moistened with KOH							
Gives off much NH <sub>3</sub> . The powder is soluble in acetic and HCl, and a crystalline precipitate formed with NH <sub>4</sub> OH.	Gives off little or no NH <sub>3</sub> . The powder is soluble in HCl and acetic acid—an amorphous precipitate falls with NH <sub>4</sub> OH.						
CaOx	Effervesces.				Gives off much NH <sub>3</sub> .		
CaCO <sub>3</sub>	Effervesces.				Gives off little or no NH <sub>3</sub> .		
Triple phosphate		Fibrin	Fat	Cystin	Xanthin		
Neutral Ca or Mg phosphate					Ammonium urate		
					Uric acid		

## THE BACTERIOLOGY OF THE URINE

The urine when examined is usually rich in bacteria of many varieties. Some of these may have been in the urine when it was voided, but the most are contaminations from the external genitalia, from the vessels which hold the urine and from the air. The urine is an excellent medium for many saprophytic organisms, and evidently for many pathogenic organisms as well. It is for this reason that, unless especial care be taken to prevent their access and growth, the urine is soon unsuitable for microscopical study. Specimens to be studied chemically should be preserved in clean bottles; chloroform, camphor, thymol, formaldehyde, etc., should be present in the bottle from the first and the specimen should be kept in an ice chest as much of the time as possible. Specimens to be studied microscopically should, whenever possible, be examined at once after voiding. And, lastly, if the specimen is to be studied bacteriologically, too great care cannot be taken to collect the urine in a way which prevents contamination.

**THE TECHNIC OF OBTAINING SPECIMENS FOR BACTERIOLOGICAL STUDY.**—It is not always or often necessary to catheterize the male patient, especially if he is intelligent enough to aid us by observing the necessary precautions. The glans penis, and especially the edges of the urethral orifice, are thoroughly washed with green soap and water, and then with bichloride of mercury (1:1000). The anterior urethra is then well irrigated with bichloride of mercury (1:60,000). The patient then voids; the most of the urine is allowed to escape, completing the irrigation of the tract, and the last few cubic centimetres are collected in a sterile test tube. A way preferred by some is to ask the patient to void into three sterile glasses. The third is the specimen examined.

It is necessary to catheterize the female patients. The external genitalia, and especially the orifice of the urethra, are well washed with green soap and water. The orifice of the urethra is then repeatedly mopped with sterile cotton pledgets soaked in sterile water, boracic acid, or mercuric chloride. At least ten or twelve of these pledgets should be used. A sterile glass catheter is then inserted with care that it touches only the orifice of the urethra. The hands of the person introducing it should be surgically clean. Over the free end of the catheter was previously fitted a rubber tube, large enough to fit loosely and about four inches long. After the most of the urine has escaped, this rubber tube is slipped off and the last small portion of urine collected in a sterile test tube. This rubber tube protects the tip of the catheter from contamination.

**BACTERIOSCOPIC EXAMINATION OF THE URINE.**—This is by far the most important part of a bacteriological examination of the urine

and should be done when cultures are also made, since by this we may get a hint as to what culture media will best serve our purpose, may discover the presence of the bacteria which will not grow on the media used, and those which have already died.

Two of the reasons why smears of urinary sediments are not oftener studied for bacteria are that it is difficult to obtain good film preparations unless all the urea, which is very hygroscopic, has been previously washed out from the sediment; and, secondly, that it is difficult to clear a urine clouded by bacteria by sedimentation or even by centrifugalization, since the specific gravity of their bodies is so nearly the same as that of the urine. And yet in the great majority of cases, especially those with even a little pus present, there is little difficulty in getting good smear preparations. One centrifugalizes the urine on a rapid machine until there is even a little sediment at the point of the tube, then quickly inverts the tube and allows all the urine to escape and drain. While still holding the tube in a perfectly vertical position a little of the sediment is scraped from the tip of the tube with a platinum loop. One must be careful to invert the tube quickly, and then while the urine is draining and while scraping the sediment with the loop not to incline it at all, else urine clinging to the sides of the tube may flow to the point and so the smear contain urinary salts.

But in case the urine is very clear and one wishes to exclude the presence of organisms, one dilutes it with one, or even two, volumes of alcohol. This will so lower its specific gravity that practically all the organisms will be thrown to the point of the tube. This fluid is very thoroughly centrifugalized, the supernatant fluid poured off, more alcohol or distilled water added, the contents of the tube well shaken, and then again centrifugalized. (There is danger that many of the organisms will be left sticking to the sides of the tube rather than be thrown to its point. To avoid this, some allow a considerable amount of the mixture of urine and alcohol to sediment in a beaker and then centrifugalize the sediment.) The sediment will now be free from urea, and satisfactory smear preparations on a glass slide or cover glass can be made. It is often wise, in case but little sediment is present, to add a little egg albumin which will stick the bacteria to the glass.

The smear preparation is first dried in the air, then passed slowly through the flame of a Bunsen burner or alcohol lamp three times, and then stained.

**BACTERIAL STAINS.**—The bacterial stains in common use are solutions of the basic aniline dyes (Grübler's are the best).

*Löffler's Methylene Blue.*—Saturated alcoholic solution of methylene blue 30 cc., aqueous solution, KOH (1:10,000) 100 cc.



The film is covered with this stain and heated over the flame for from one to five minutes. When no heat is used the staining will take much longer. The stain is then washed off with water, the film dried with blotting or filter paper, and then mounted in Canada balsam. If on a slide the smear may be studied without the interposition of a cover glass.

*Saturated Aqueous Solution of Methylene Blue.*—This is used as the above, but stains a little more slowly.

*Aniline Gentian Violet.*—Aniline oil water is first made by adding exactly 2 cc. of aniline oil to 98 cc. of distilled water in a flask. This is shaken vigorously till as much as possible of the oil is dissolved, and filtered twice through the same paper. This fluid is kept in a dark-glass bottle and in a dark place.

To 75 cc. of this aniline oil water are added 25 cc. of a saturated alcoholic solution of gentian violet, and the mixture filtered. This staining mixture is fairly permanent, but should not be exposed to strong sunlight and should be occasionally filtered. Smears will stain readily in this in a few minutes. This is the stain used in Gram's method (see page 89).

*Piffaud's Method of Staining Bacteria* (*N. Y. Med. Jour.*, Nov. 2, 1907).—This is a valuable method for determining the nature of a growth.

Cyanide blue solution:

Distilled water 100 parts  
Potassium cyanide (pure) 1 part  
Potassium carbonate (dry; pure) 0.5 part  
Rectified methylene blue 0.5 part

A small drop is placed on the centre of a slide, and then a loop of the growth well mixed with it. After one minute a clean cover glass is dropped on, and the excess of the moisture absorbed by pressing the cover glass firmly with a piece of filter paper. In this way one dispenses with drying, heating and long staining.

*Carbolfuchsin.*—

Basic fuchsin 1 part  
Absolute alcohol 10 parts  
Carbolic acid solution (1:20) 100 parts.

This is a very powerful stain. When used undiluted from one-half to one minute's staining is sufficient, but better results are obtained when it is diluted with from 5 to 10 volumes of water and left in contact with the smear for a few minutes. This is the stain used for the tubercle bacillus (see page 50).

*Bismarck Brown* (see page 66).

*Staining Methods for Acid-Fast Bacilli* (see page 50).

*Gram's Method* (see page 89).

*Capsule Stains* (see page 62).

**SPORE STAINING**—The film is first placed in chloroform for two minutes and then well washed in water. It is next placed in a 5 per cent. solution of chromic acid for from one-half to two minutes, and again well washed with water. It is then stained with carbolfuchsin while heating in the same manner as if one were staining the tubercle bacillus (see page 50). The carbolfuchsin is not washed off in water, but with 1 per cent. sulphuric acid, or with methylated spirit (Ethyl alcohol 9 parts, methyl alcohol 1 part), and left in this until decolorized. It is then washed in water, stained with a saturated aqueous solution of methylene blue for half a minute, washed again in water, dried, and mounted in balsam. The spores will retain the red stain, the bacilli will take the blue.

**FLAGELLA STAINING.**—It is very difficult to get good results since only under certain conditions of growth, age, etc., can the flagella be demonstrated, and even when the culture is a very suitable one, the flagella may be very easily injured by technic. The smear should always be made from a young agar culture, incubated at 37° C. for from 12 to 18 hours. Kendall recommends to inoculate gently 5 cc. of sterile water with enough of the above-mentioned growth to produce a faint turbidity in the upper half of the tube. This tube is then placed in a thermostat for one hour. This will allow the clumps to settle and the organisms to multiply a little. Without disturbing the fluid any more than one can help, two or three

loopfuls are removed and placed on a clean cover glass without spreading and dried in a thermostat. The specimen is then fixed in a flame. (The cover glass should be one which has been washed in a mixture of concentrated sulphuric acid, 6 parts, potassium bichromate, 6 parts, and water, 100 parts. It should then be washed thoroughly in water, and stored in absolute alcohol.)

The staining methods are all of them so unsatisfactory that the best is usually the one with which the worker is most familiar. (For Löffler's and other methods see Muir and Ritchie, *Manual of Bacteriology*, 1903, American Edition.)

*Pitfield's Method as Modified by Richard Muir.*—

The mordant consists of:

Tannic acid, 10 per cent. aqueous solution, filtered, 10 cc.

Alum, saturated aqueous solution, 5 cc.

Corrosive sublimate, saturated aqueous solution, 5 cc.

Carbolfuchsin stain (see page 283), 5 cc.

This is mixed thoroughly. A precipitate forms which is allowed to settle, or the fluid is centrifugalized, and the clear supernatant fluid removed with a pipette and kept in a clean bottle. This will keep for one or two weeks.

The stain is,

Alum, saturated aqueous solution, 10 cc.

Gentian violet, saturated alcoholic solution, 2 cc.

This should be not more than 2 or 3 days old when used.

The film prepared as described above, is covered with as much of the mordant as the cover glass will hold, heated over a flame and allowed to steam gently for about one minute. It is then well washed in running water for about two minutes. It is then carefully dried over a flame, then covered with the stain, heated, allowed to steam for about a minute, washed well in water, dried and mounted.

Study of the smears of the sediment will give some clew as to the presence of organisms and the nature of some of those seen. For some organisms it is the best method of study we have. This is especially true of the tubercle bacillus and streptococci.

BACILLUS TUBERCULOSIS (for staining methods see page 50) may be found in the urine in cases of tuberculosis of the kidney or of any portion of the urinary tract, providing the tuberculous focus is discharging into this tract. The disease may be extensive in a kidney, *e.g.*, but if a focus has not ulcerated into the pelvis of the kidney, or if that kidney is not secreting any urine, no tubercle bacilli will be found in the urine. But tubercle bacilli are not infrequently found in the urine of patients with miliary tuberculosis,\* and some think they can be found in the urine of patients with pulmonary tuberculosis, or in fact with tuberculosis of any organ, and that their presence does not indicate local tuberculosis of the urinary tract unless pus also is present in the urine, or there are other signs of local tuberculous disease. Cases of sterile pyuria are often due to tuberculosis.

*The Smegma Bacillus* is an organism which grows in abundance on the external genitalia and wherever the secretions of the skin accumulate. Its morphology and staining characteristics vary somewhat. Some of its strains closely resemble the tubercle bacilli, both in morphology and in its acid- and alcohol-fast staining reactions, so

\* Churchman. *Am. Jour. Med. Sc.*, July, 1905.

that the only sure method of differentiation is animal inoculation. Twenty-one different stains have been published to differentiate these organisms from the tubercle bacillus, but in vain. It is not necessary to differentiate between them. It is much easier to avoid them entirely by cleanliness in obtaining the specimens, and then any acid-alcohol-fast bacillus can safely be called *Bacillus tuberculosis*.

*Streptococci* also are much more safely searched for in the smears from the sediment than by cultural methods, and no matter what the organism is, one should always control his cultures by a preliminary bacterioscopic examination.

#### THE BACTERIOLOGY OF THE URINE, CULTURAL METHOD

The last portion of the urine voided is well centrifugalized, without the addition of alcohol (see page 284), or sterile water may be added to dilute the urine and to wash the sediments, and cultures are made from the sediment.

The culture medium used will depend in great measure on the organisms suspected. When, however, the nature of the organism is not known, blood agar is the best medium to use, since all organisms which can be cultivated will grow on this. A few loopfuls of the urinary sediment are rubbed over the surface of this medium and the tubes then inoculated at 37° C. The different colonies can then be distinguished and transplantations to suitable media be made.

The MEDIA in common use are the following:

*Nutrient Agar*.—About 1500 cc. of distilled water are heated slowly over a furnace in a large metal pan. Meanwhile 15 gm. of agar are shredded and added, together with 2.5 gm. of Liebig's meat extract. The heating is continued, stirring at intervals until all the agar is dissolved. All floating scum is skimmed off with a spoon. The pan is then removed from the fire, cooled slightly, and 10 gm. of peptone (Witte's) and 5 gm. of sodium chloride added slowly, little by little, stirring vigorously all the while to facilitate solution. The pan is then replaced on the fire and the contents boiled and stirred until all the peptone is dissolved. The reaction of the fluid is then made just alkaline (to litmus) by the addition of a 5 per cent. solution of sodium hydrate. The pan is then removed from the fire and cooled to 60° F. In the meanwhile the whites of two eggs have been mixed with 150 cc. of water. This is added to the agar solution, the pan then replaced on the furnace, and slowly heated until coagulation is complete. The fluid is not stirred during this coagulating process. When coagulation is complete, the pan and contents are weighed, and enough water added to bring the weight of the contents to just 1000 gms. (Twenty grams are allowed for the weight of each white of egg which will be filtered off.)

Meanwhile a large funnel with rubber tube and pinch-cock on the nozzle has been set up on a retort stand. In this is placed a well-moistened filter paper folded in a wire holder. The contents of the pan are now poured into the funnel through a strainer which will remove the coagulum. The medium is filtered at once into tubes or into a flask and sterilized for seven minutes in an autoclave.

If the medium filters too slowly, it is poured back into the pan, reheated, and filtered through a fresh filter paper.

This is the medium most used for the ordinary specimens and also is the basis of other special media.

*Glycerin Agar.*—This medium is similar to the above except that to it after filtration is added from 6 to 8 per cent. of glycerin. The medium is then tubed and sterilized in the autoclave for seven minutes. This medium is superior to plain agar (the above medium) since many organisms which grow delicately on the latter will grow well on this. This is true of streptococci, the meningococcus, the pneumococcus and the tubercle bacillus.

*Blood Agar.*—This is one of the most valuable of media.

*Rosenau's Method.*—About one hundred sterile slant tubes of plain agar are first prepared and then a flask containing 50 cc. of plain agar which is melted at from 40° to 60° C. Just 15 cc. of human blood are added under aseptic precautions to the 50 cc. of melted agar, the contents of the flask well mixed by shaking, and from 1 to 2 cc. of this are poured into each of the slant agar tubes. The tubes are then placed in the proper position that this agar-blood mixture may harden as a uniform layer over the plain agar slant. The technic must be aseptic to prevent contamination of the tubes, since they must not be sterilized again. All the tubes are then left in the thermostat for a few days to be sure they are sterile. If made in this way a little human blood will make a great many tubes.

On this medium will grow practically all organisms which can be cultivated at all. It is especially good for the gonococcus and *Bacillus influenzae*.

*Nutrient Gelatin.*—This is made in practically the same way as is nutrient agar, with the substitution of from 100 to 150 gms. of "gold leaf" gelatin for the agar. This medium should, however, be boiled as little as possible and should be sterilized in the autoclave for but five minutes. (Of course tubes of this medium should not be placed in a thermostat kept at body temperature.)

*Litmus Milk.*—About 100 cc. of fresh milk are allowed to stand in the refrigerator for about five hours and as much of the cream as possible removed. Then enough litmus tincture is added to give the milk a deep sky-blue color. The medium is then tubed and sterilized in the autoclave for seven minutes.

*Bouillon.*—The formula is:

Liebig's meat extract, 2.5 gm.

Peptone (Witte's), 10. gm.

Sodium chloride, 5 gm.

Water, distilled, 1000 cc.

The steps in the making of this medium are practically the same as those for plain agar. To the boiling water is added the meat extract and the boiling continued for five minutes. The pan is then cooled down a little, the peptone and salt added slowly and then dissolved by boiling, and the fluid made alkaline as described above. This medium when finished is first poured into a flask and sterilized in an autoclave for five minutes, then cooled, filtered twice through the same paper, then tubed and sterilized again in the autoclave for five minutes.

*Löffler's Blood-serum Mixture.*—See page 88.

Among the more important ORGANISMS which may be encountered in the urine are the following. We give a few of the important features of each and for more detailed description would refer the reader to any standard text book of bacteriology.

*Bacillus Coli Communis.*—The colon group includes twenty or thirty very similar varieties, which usually are grouped under this one name. It is a short organism, the majority from 1 to 2  $\mu$  long and 0.5  $\mu$  thick. Some, however, are so short as to resemble cocci and others are over 5  $\mu$  long. It often occurs in pairs. It is a sluggishly motile bacillus, although its motility is not always evident and often cannot be demonstrated at all in cultures over 24 hours old. Some strains, however, are very motile. Its flagella are numerous and laterally placed. It stains well by all the usual aniline dyes and decolorizes by the Gram method. It does not produce spores. The organisms with unstained portions resembling

spores are involution forms. It grows rapidly on all media at room as well as at body temperature, and in a characteristic way. The growth on the surface of agar is abundant, thick, moist, and spreads rapidly. The deep colonies are very circumscribed and opaque with a well developed nucleus. This bacillus does not liquefy gelatin and does not spread on the surface of this medium as on agar. The surface colonies on gelatin have a nucleus and well-defined granular or striated refractive halo. It turns litmus rapidly (within 18 hours) acid, coagulates it (in from 4 to 30 days) and does not later digest the clot. The growth on potato is abundant and visible. It ferments almost every carbohydrate known, but especially glucose, lactose, and saccharose, with abundant gas production. It produces indol. The colon bacillus is almost ubiquitous. It is the prevailing organism of the lower part of the small intestine and the colon, and is almost universal in soil, water, food, etc. It is a mildly pathogenic and pyogenic organism. It is the organism found most frequently in infections of the urinary tract.

*Bacillus Typhosus*.—The typhoid bacillus is a long slender organism measuring usually from 2 to 4  $\mu$  in length and about 0.5  $\mu$  in thickness. It is actively motile. Its flagella are more numerous (from 5 to 20) and somewhat longer than those of *Bacillus coli communis*. It stains easily by all the aniline dyes and decolorizes by Gram's method. It does not produce spores. It grows on all ordinary media. The growth on agar is thin and translucent with slightly spreading dentate or leaf-like edges. The deep colonies have sharply defined edges and a distinct nucleus. *Bacillus typhosus* does not liquefy gelatin. Its growth on potato is often, but by no means always, invisible. (The colon bacillus grows on potato as a distinct brownish scum, and the typhoid bacillus may also. This seems to depend on the potato used.) A most important feature is the reaction of this bacillus on litmus milk. A very slight acidity is first produced, distinct, but never enough to coagulate the milk not even in weeks. This slight acidity may be permanent although some strains later furnish enough alkali to change the reaction back to neutral, or even alkaline. Certain carbohydrates, including dextrose, levulose, maltose and mannites are fermented to the point of acidity, but none with gas production. Saccharose and lactose are not at all affected. It does not form indol.

For the certain recognition of this bacillus, its agglutination in the very dilute serum (1:50 to 1:1000 even) of a patient or animal immune to *Bacillus typhosus* is necessary (see page 299).

This organism is often present in the urine, in even over one-third of all cases (see page 299) during typhoid fever, and in some cases for years afterward.

*The Paratyphoid Group of Organisms*.—The more carefully are cases of "typhoid fever" studied bacteriologically, the more numerous are found to be the cases due not to *Bacillus typhosus* but to bacilli very similar to it, but resembling in some essential respects *Bacillus coli communis*. Those which stand nearest this organism are called paracolon bacilli. There is no one *Bacillus paratyphosus*, but a group. In the clinical laboratory of the Johns Hopkins Hospital are kept at least eleven different strains of this organism. They seem to stand between *Bacillus typhosus* and *Bacillus coli communis*, but the court of last appeal is the serum agglutination test, which is a fairly satisfactory means of differentiation, although they show in some cases a rather marked group reaction.

The characteristic cultural features of the paratyphoid bacilli are their reaction to milk and to sugars. All resemble *Bacillus typhosus* in that they produce first a slight acidity in milk. Then all (and this may take weeks) finally change the reaction of the milk back to alkaline. The attempt to divide the group into the A group of organisms which keep the milk acid and the B group which change it back to alkaline, seems unjustified (Ford, *Medical News*, June 17, 1905). And they all ferment glucose with gas production. Some ferment also lactose, some saccharose, but none all three sugars. But the court of last appeal is their reaction to the serum of animals immunized to one of the strains of these bacilli. This is fairly specific, although they do show a group reaction.

*Bacillus Lactis Aërogenes*.—This is a short thick (about 2  $\mu$  long and 0.7  $\mu$  thick) non-motile, encapsulated bacillus. It occurs usually in pairs and sometimes in

chains. It often shows rather marked polar staining. It is decolorized by Gram's method. It is not a spore-producing organism. It produces on all media a luxuriant viscid, slimy growth which resembles in many ways that of *Bacillus coli communis*. It does not liquefy gelatin. It ferments practically all the carbohydrates rapidly and with abundant gas production. This organism has been the subject of much dispute. Some do not distinguish it from *Bacillus coli communis*, some group it with the capsulated group. Some strains of this organism cannot be distinguished from *Bacillus capsulatus* of Friedländer (*Bacillus pneumoniae*).

This bacillus is a normal inhabitant of the upper part of the small intestine, where it is the predominating organism. Some believe it the cause of certain cases of cystitis.

*Bacillus Alkaligenes*.—This bacillus is a normal inhabitant of the intestinal tract. Its greatest importance is that it is often mistaken for *Bacillus typhosus*. It is a long, slender bacillus from 2 to 3  $\mu$  long and 0.5  $\mu$  thick, which grows singly, in pairs, or in chains. It is actively motile.

It grows on all culture media. It does not liquefy gelatin. Its most characteristic cultural reaction is the production of an intense alkaline reaction in litmus milk without previous acid production. It ferments carbohydrates without producing an acid reaction and without gas production, and is the only one of the common intestinal flora which grows only in the open end of the fermentation tubes, and not at all in the closed end.

*The Proteus Group*.—The members of this group, among which are *Proteus vulgaris*, *Proteus mirabilis*, *Proteus Zenkeri*, *Proteus Zoffii*, and other strains with but minor differences, are the most important agents of putrefaction. While secondary invaders as a rule, in certain cases of cystitis they are the pathogenic organism. In fact this is almost the only organism which when introduced into a normal bladder can set up cystitis. They are the important organisms in the production of "sapræmia," or intoxication from the products of decomposition, as from a portion of retained placenta. They are short, slender, actively, even violently, motile bacilli with terminal flagella. This description of the organism applies only to fresh cultures. If those a little older be examined, one will find in the smears cocci, bacilli of all sizes, spirilla, etc. The smear will suggest a badly contaminated culture. But if fresh transplantations be made and examined early, the organisms will be found to be bacilli of uniform morphology. The above polymorphism is explained by involution forms. It is decolorized (?) by Gram's method. It grows well at room temperature. The colonies on agar spread rapidly in a characteristic manner, the edges sending out peculiar, hair-like projections, and look like tufts of moss. *Proteus vulgaris* and *mirabilis* liquefy gelatin (the former rapidly, the latter slowly) and blood serum rapidly. *Proteus Zenkeri* and *Zoffii* do not. Milk is coagulated and the clot then digested with the reaction alkaline. The strains differ somewhat in their ability to ferment sugars. The formerly much talked-of *Bacterium termo* is possibly one of this or of a nearly related group.

*Bacillus Pyocyaneus*.—*Bacillus pyocyaneus* is a small organism about 2  $\mu$  long and 0.5  $\mu$  thick, actively motile, and which stains well in all ordinary bacterial stains, showing often a marked bipolar staining. It decolorizes by Gram's method. It grows rapidly on all ordinary media and will crowd out any other organism which happens to grow along with it. Its characteristic cultural characteristics are, that it will not split up carbohydrates, that it liquefies gelatin and blood serum rapidly, that it coagulates litmus milk rapidly, decolorizing the litmus and then digesting the milk clot with the reaction alkaline, and lastly that it produced two pigments in its growth,—a non-specific fluorescent pigment and a specific bluish-green pigment called "pyocyanin."

This is a very common organism, occurring often in the intestine, and on the skin, especially in the folds of the axillæ and groin. It is the pyogenic bacillus which produces blue pus. It is the organism of some cases of septicæmia of children, but its most important rôle, as a pathogenic organism, is as the cause of cystitis and ascending genito-urinary infections.

*BACILLUS AEROGENES CAPSULATUS*.—This is one of the most widely distributed

organisms known. It is a constant inhabitant of the intestine of men and animals, and is common in the soil, water, milk, etc.

This is a large bacillus, from 1.5 to 6  $\mu$  long and 1  $\mu$  thick. It is non-motile, capsulated, and produces spores in the animal body and when grown on blood serum. It stains easily in all the ordinary stains and does not decolorize by Gram's method.

*Bacillus aerogenes capsulatus* is a pure anaërobe, growing only in the complete absence of oxygen. It grows best (under anaërobic conditions) at 37° C. in the depth of solid media as grayish-white or brownish colonies with fine feathery or hair-like projections from the edges of the colonies. It ferments sugars easily. Litmus milk is coagulated, decolorized, and the clot later digested.

Since it occurs very often in mixed infections the best way to cultivate it is to heat the specimen containing the mixture of organisms at 80° C. for a few minutes to kill off all but spores. Then plates are made which are cultivated anaërobically. (The most of organisms in a mixture are not spore producers, and so after the heating but few forms will be left alive from which to select this bacillus.)

A still better way is to inoculate a rabbit intravenously with the material containing the mixture of organisms. After five minutes the animal is killed and placed in the thermostat for 6 or 8 hours or left in a room temperature for 18 to 24 hours. The animal will become much distended with gas. *Bacillus aerogenes capsulatus* can now be obtained in almost pure culture from the blood.

This organism is one of the most important of the pathogenic bacteria, and is yearly assuming greater importance. Infections with this organism are extremely grave. Those of the genito-urinary tract occur not infrequently as the result of dirty technic in obstetrics.

**BACILLUS TETANI.**—The tetanus bacillus is a slender organism about 4 or 5  $\mu$  long and 0.5  $\mu$  thick. It is a motile bacillus and has a great many very long slender flagella. It is a spore producing bacillus and since the spores have a diameter three or four times that of the bacillus and are usually situated at the end of the bacillus the shape of the sporulated organism is characteristic, resembling a drum-stick. Sometimes there will be a spore at each end and the result is a dumb-bell form. These spores are very resistant to heat, and are not killed by a temperature of 80° C. for one hour.

This bacillus stains well in all the ordinary bacterial stains, the body of the bacillus taking an unusually uniform stain. It is not decolorized by Gram's method.

The tetanus bacillus is a perfect anaërobe (grows only in the absence of oxygen) and can be isolated only with extreme difficulty. Fortunately it is not necessary to grow it since its appearance in smears is characteristic.

This organism is one of the most ubiquitous and important of pathogenic organisms. Its normal habitat seems to be the intestine of cattle and so it is widespread wherever is contamination with manure.

*Staphylococcus Pyogenes Aureus.* (*Micrococcus aureus*.)—This organism is a coccus which occurs in groups, hence the name "staphylococcus." When actively growing it may occur singly or as diplococci. The individual organisms are spheres a little less than 1 micron in diameter. It stains well in all aniline dyes and is not decolorized by Gram's method. It is not a flagellated coccus. It grows well in all ordinary media. If grown directly from the animal body its colonies will sooner or later develop a golden yellow pigment, seen first at the edges of the thick, glistening, dull white growth, and later spreading throughout the whole growth. The most pigment is produced on potato. This organism liquefies gelatin, produces acid in milk, and ferments with gas production nearly all sugars. It is pathogenic to animals, producing either a fatal septicæmia, or, if the animal survives for a few days, a pyæmia with multiple abscess formation. In man it is the common pus producing organism. It especially produces local pus foci; as boils, abscesses, etc.

*Staphylococcus pyogenes albus* is also one of the most important pyogenic



organism. It differs from *Staphylococcus pyogenes aureus* only in that no yellow pigment is produced. Many think that it is really a variant strain of aureus.

*Staphylococcus epidermidis albus* is a normal inhabitant of the deep layers of the skin. It is quite similar to *Staphylococcus pyogenes albus*, but is less pathogenic and a feebler grower.

*Streptococcus Pyogenes*.—This organism is a coccus about  $1\ \mu$  in diameter which occurs in chains of from two to one hundred or more. The members of the chain are slightly flattened against each other. It is not a capsulated streptococcus as are the chain forms of *Diplococcus lanceolatus* and as is *Streptococcus*



FIG. 54a.—*Micrococcus aureus*. Photomicrograph by Dr. Thomas M. Wright.

*mucosus*. It does not decolorize by Gram's method. It grows on all ordinary media, but is a feeble grower, and its colonies are so minute and translucent that they are easily overlooked. Also this organism is quickly overgrown in mixed cultures. That there are several varieties of streptococci none will deny, but observers cannot agree on any manner of division of the groups. Some strains of streptococci do not grow at all at room temperature; some coagulate milk, others do not; some grow on gelatin, which they do not liquefy, others do not; all are quickly killed off in acid-reacting media, and all decolorize litmus milk.

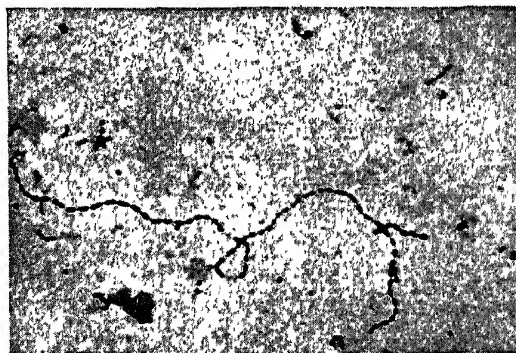


FIG. 54b.—*Streptococcus pyogenes*. Photomicrograph by Dr. Thomas M. Wright.

In the search for this organism in the urine, sputum, pus, etc., bacterioscopy of the exudate is of far more value than cultural methods.

*Streptococcus pyogenes* is an intensely pathogenic organism in man, producing rapidly spreading inflammations with much necrosis, but with little pus production. It is especially prone to cause inflammations of the serous membranes. It is an important cause of septicæmia and pyæmia. It is the most widely spread of all pathogenic organisms.

THE BACTERIOLOGICAL STUDY OF THE URINE is often important in septicæmia, perhaps in acute nephritis due to infection of the renal



cortex, certainly in pyelitis, ureteritis, cystitis, prostatitis and urethritis.

In cases of SEPTICÆMIA one seldom searches for the organism in the urine, since blood cultures are much more satisfactory. But since the importance of typhoid bacilluria in the spread of that disease has been recognized, the presence of this organism in the urine has aroused a new interest. It is now known that *Bacillus typhosus* can be found in the urine of about one-third of the cases of typhoid fever during the attack. Sometimes they are few in number, sometimes so many that the urine is clouded by them alone and shimmers when shaken as does a bouillon culture. Even five hundred million of these bacilli may be found in one cubic centimetre of the fresh urine. While as a rule this bacillus clears up with the fever, yet in certain cases it may persist for years, and few or no local symptoms warn these "chronic bacillus carriers" that they are spreading an epidemic of typhoid fever far and wide. It is because of this bacilluria that all typhoid patients are now given urotropin during their convalescence. These organisms must have come from the blood originally, but that they later multiply in the pelvis of the kidney seems most reasonable.

In other forms of septicæmia the invading organism is often found in the urine. The occurrence in the urine of *Bacillus tuberculosis* in cases of chronic tuberculosis and especially of the acute miliary form has already been mentioned. In a recent case of streptococcus meningitis secondary to otitis media and mastoiditis, the fresh urine on the day of death attracted attention because it was so cloudy. Microscopical examination showed that the turbidity was due entirely to chains of streptococci.

That the bacteriology of the urine in acute infectious diseases will assume greater and greater importance there can be little doubt. It was the opinion some years ago that in acute infectious diseases the kidneys "excreted" the organisms, but animal experiments did not bear out this opinion, and so for a few years they were supposed to be killed and disposed of by the tissues. Now the question again assumes importance, since in typhoid fever at least the output of the bacilli in the urine is something enormous.

INFECTIOUS NEPHRITIS.—So many cases of acute nephritis date to an acute infectious disease, such as pneumonia, tonsillitis, influenza, typhoid fever etc. (to say nothing of scarlet fever, measles, etc., diseases the specific organisms of which are not known) or to an acute infection, as in a recent case of streptococcus infection of the arm with acute nephritis developing while under observation, that it would not be unreasonable to think that the complicating acute nephritis might be due to the same organisms that cause the primary infection, and that cultures from the urine of these cases might lead to interesting results. The same may be true of the so-called "febrile" albuminuria.

The interesting cases of unilateral nephritis certainly suggest a local infection of the renal cortex, and, clinically, there is a group of chronic nephritis cases which run a quite different course from the usual (see page 324). This subject has received very little attention.

ACUTE PYELITIS is practically always an infection. Fortunately now it is an easy matter, thanks to the gynecologists and genito-urinary surgeons, to catheterize the ureters of women and men, and so to get cultures directly from the pelvis of each kidney. Acute pyelitis may be due to a primary infection of a normal kidney by organisms in the blood, or to an infection secondary to the irritation of a stone, *e.g.*, to an ascending infection. In a pyuria of renal origin it is often easy to cultivate the infecting organisms. If the urine cultures are sterile, the cases are usually of tuberculosis of the kidney. We will speak of the bacteriological findings in pyelitis in connection with those in cystitis.

It may be mentioned here that pyelitis without localizing symptoms is quite common, and that a pyelitis may continue for considerable time before a cystitis begins.

CYSTITIS.—Among the cases of cystitis those due to tuberculosis form so well defined a group that they are here described separately.

*Tuberculous Cystitis.*—A primary tuberculous cystitis is a very rare condition. The disease usually descends from the kidney, but in men may also ascend from the genital tract. The diagnosis of the descending cases especially is of great importance, since the symptoms are usually vesical, and the renal disease even though of extreme grade may pass unnoticed both by patient and doctor. The question of renal tuberculosis can best be decided by cystoscopic examination, and, if necessary, ureteral catheterization, which will decide the source of the pus. In the following pages we include under the urinary findings of cystitis those which the kidneys contribute.

Cystitis is an inflammation of the bladder due to some infecting organism. The normal bladder is very resistant, and therefore hard to infect. Organisms introduced are quickly gotten rid of, and seldom set up an inflammation. This resistance to infection seems explained by the nature of the bladder's epithelial lining. Most organisms can gain foothold there only after some predisposing factor has lowered the resistance of the bladder wall. Among these conditions favoring infection may be mentioned, the irritation from a calculus; a posterior gonorrhœal ureteritis; and especially the retention of urine from any cause, such as childbirth, enlarged prostate, urethral stricture, spinal cord disease, and prolonged narcosis. If to these conditions be added frequent catheterizations the chances of a cystitis are excellent. There is another origin of infection which just now is attracting considerable attention; we refer to a direct extension from the rectum. Animal

experiments would suggest that disease or injury of the rectal mucosa is an important element in this infection. There are two organisms which seem exceptions to the above rule that the resistance of the bladder wall must first be considerably lowered,—*i.e.*, the tubercle bacillus, and the proteus. The latter organism if introduced into a normal bladder will set up a cystitis.

Tuberculous cystitis occurs especially in young patients. It begins insidiously, and lasts for years. There is usually increased frequency of micturition, but not always. The urine is acid; there is a variable amount of, and often considerable, pus present, and cultures made from the urine are negative. The pyuria may have escaped the patient's attention and the condition be accidentally discovered. There are two general rules. All persistent, acid pyurias in the young are presumably tuberculous until the contrary is proven (Kelly); and all sterile pyurias are of either tuberculous or gonorrhœal origin. The tubercle bacilli should be looked for and usually will be found (see page 292). It is easier to demonstrate the tubercle bacillus in tuberculous cases than the gonococcus in the gonorrhœal.

There is another interesting group of very early cases of tuberculous cystitis in which practically no pus is present in the urine. There is slightly increased frequency of micturition, and hæmaturia. The hæmaturia is slight and transitory. It may persist for a few days and then not reappear until after an interval of weeks. The urine is clear, rather highly colored, but with the last of the voiding are usually seen a few drops of blood.

In advanced cases of tuberculous cystitis the urine remains acid, there is often much pus, tubercle bacilli are easy to demonstrate, and there is persistent or recurring hæmaturia. Following a secondary infection, and this is sooner or later quite certain to follow, the pus usually increases and the urine often becomes alkaline.

It is important to remember that cases of tuberculous prostatitis and ureteritis may so resemble tuberculous cystitis that only a cystoscopic examination will decide the question.

*Cystitis due to Organisms other than Bacillus Tuberculosis.*—Among the organisms found are *Bacillus coli communis*, *Staphylococcus pyogenes aureus*, *Staphylococcus pyogenes albus*, *Streptococcus pyogenes*, *Bacillus proteus vulgaris*, *Bacillus pyocyaneus*, *Bacillus typhosus*, *Bacillus lactis aërogenes*, and others. In many cases it is difficult to determine just how important in the etiology of the cystitis the organism found is.

Many organisms found are certainly harmless saprophytes.

*Bacillus coli communis* is the commonest invading organism. This sets up a very chronic cystitis.

The gonococcus can with great difficulty get a foothold in the bladder, for in all the very numerous cases of gonorrhœal posterior urethritis the organism must enter the bladder. The wall at the trigone seems the most susceptible spot in the bladder. A definite cystitis may develop as part of an acute urethritis and clears up rapidly, unless we include most of the cases of acute trigonitis. The gonorrhœal and tuberculous types are the two which seem exceptions to the rule that pyuria is an almost invariable symptom of cystitis. It is very difficult indeed to demonstrate the gonococcus in cystitis. Secondary pyogenic infections superimposed on a gonorrhœal cystitis are a not uncommon sequel of this condition.

*Proteus* cystitis is a common and very distressing form. This seems almost the only organism which when introduced into a normal bladder will set up a cystitis (Melchior). The urine in these cases is very alkaline, and the abundant pus is transformed into a ropy, sticky, mucoid material.

The streptococcus cystitis is often a very severe form, but sometimes is mild. *Bacillus lactis aërogenes* is said to be a much commoner cause of cystitis than the figures would lead one to expect.

The catheterized urine in cystitis practically always contains pus. Certain cases of tuberculosis and of gonorrhœa are possible exceptions to this rule. As already mentioned, the source of much of this pus may be the kidney. When there is fever a pyelitis should be suspected; also when there is more albumin than the pus alone could explain. The pus varies greatly in amount. As a rule the most of the pus appears in the first glass, and least in the second glass of the three-glass test. This holds true only if the patient has been resting before voiding. The condition of the pus cells will depend on the reaction of the urine. When this is acid, the pus cells are well preserved, sometimes amœboid, and settle as a granular layer on the bottom of the glass. When very alkaline, as in *proteus* cystitis, not one pus cell may be seen. The pus is transformed into a sticky, ropy, mucoid substance.

The reaction of the urine in the tuberculous cases is usually acid until secondary infection by *proteus* or the streptococcus occurs. In chronic cystitis due to the colon bacillus and *Staphylococcus albus* and other organisms with slight virulence the urine may be acid or alkaline. When the urine is alkaline the phosphates are precipitated, the pus cells transformed and the odor becomes alkaline and foul.

Red blood cells are numerous in the urine of cases of acute cystitis and vary in number with the acuteness of the attack. They are uniformly mixed with the urine. The hæmorrhages from the bladder wall are slight. The larger hæmorrhages come from the kidney, from vesical tumors, or especially from the prostatic urethra. In the posterior urethritis cases blood may continuously ooze back into the

bladder and the voiding of bloody urine be followed often by a little pure blood.

For mention of the epithelial cells, see page 265.

In "membranous," "exfoliative," "croupous," "diphtheritic," or "desquamative" cystitis the patient passes flakes, or masses, or moulds of a tough fibrinous membrane containing much degenerated epithelium. These are supposed to be due to necrosis of the inner layers of the bladder wall. In gangrenous cystitis fragments of the epithelial and muscular coats of the bladder are expelled in the urine. In hemorrhagic cystitis there may be much bloody infiltration of the bladder wall. It might be mentioned that one sees clinically these severe forms very seldom. They occur especially after traumatic or operative opening of the bladder and are usually terminal events.

An interesting problem for the clinical laboratory workers to decide is how well justified is the statement, frequently heard, that changes in the reaction, the specific gravity, and the amounts of normal constituents of the urine are important in lowering the resistance of the wall of the bladder to infection.

BACTERIURIA.—By bacteriuria is meant the presence in the urine when voided of so many organisms that they cloud the urine. We mention this subject here since there is usually a mild cystitis or later there will be, and since the urinary features indicate such a condition. Indeed the symptoms may indicate a severe cystitis and yet cystoscopic examination show the bladder normal.

A *transitory bacteriuria* often follows massage of the prostate gland. It begins within a few hours after this procedure and may last one or two days. There are no symptoms. The organisms are supposed to come originally from the rectum.

The cases of *persistent bacteriuria* fall into two groups. The first of these is of renal origin, and has been already mentioned on page 299. The organisms in these cases are usually either the typhoid or the colon bacilli. The second group includes the cases secondary to a posterior urethritis and prostatitis. The foci of infection is in these organs, but the organisms grow in the bladder. They are so numerous that the urine is very cloudy. Very little pus is present.

In gonococcus, typhoid, and colon bacteriuria the urine remains acid. In streptococcus and staphylococcus bacteriuria the reaction may be acid, neutral, or alkaline. The staphylococcus albus bacteriuria, however, is usually associated with alkaline urine.

#### INFECTIONS OF THE URETHRA AND EXTERNAL GENITAL ORGANS

In this connection it will be necessary to describe three very important organisms not mentioned in the preceding pages.

**THE GONOCOCCUS.**—The gonococcus is an organism deserving special mention. Every clinical laboratory worker should be skilled in its recognition. Formerly supposed to be the cause of unimportant, transitory, local infections, it is now known to be one of the most important of organisms.

The gonococcus (see Fig. 54c) is a coccus about  $1\ \mu$  in diameter, which occurs, as a rule, in pairs. The adjacent edges of these diplococci are flattened, giving the organism the well known biscuit shape. In smears of gonorrhoeal pus it is found chiefly inside pus and epithelial cells, or in masses on their surface; but some are found free. It stains well in all the ordinary bacterial stains, but especially in methylene blue, and discolorizes by Gram's method. (This organism in smears of pus, however, decolorizes so slowly that it may be necessary to leave the specimen in alcohol for ten minutes.) It grows only on media containing some albuminous constituent of the human body. For its recognition it is necessary to show that it occurs as biscuit-shaped diplococci, in clusters or clumps, some of them at least intracellular, that it decolorizes by Gram's method, and that it grows only on the above mentioned media. Blood agar and blood serum are excellent media, but agar mixed with ascitic fluid or hydrocele fluid is good, or a medium made up of urine, blood serum and agar. Its growth on proper media is a thin moist homogeneous layer which dies out in a few days. Cultivated under the best

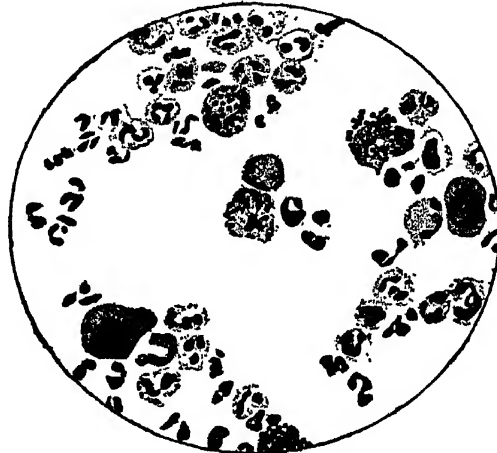


FIG. 54c.—Spread of pus containing gonococci. (Wilson.)

conditions and transplanted frequently, the gonococcus will survive but for a few generations. It is very susceptible to changes in temperature. It dies in a few hours in pus at room temperature, and is quickly killed by a temperature of  $40^{\circ}$  or  $41^{\circ}$  C. It is killed rapidly by drying.

The gonococcus is the important causative agent for urethritis and periurethritis, prostatitis, and infection of the connecting ducts and glands of the genito-urinary system, the seminal vesicle, prostate, epididymis, bladder, Bartholin's glands, vagina, uterus, tubes, etc.; it causes an eczematous skin eruption about the genitals; it is an important cause of proctitis, peritonitis, meningitis, endocarditis, and especially of conjunctivitis, arthritis, and septicæmia. In all these conditions the clinical laboratory worker must be on the lookout for the gonococcus. One should not demand a discharge as a necessary indication of possible gonorrhoeal infection, for it may be found where little pus is present, as in the vaginal discharge of infants, and the fluid from the joints with chronic arthritis.

Much has been written of the organisms often found in the normal urethra which morphologically cannot be told from the gonococcus, and which also decolorize by Gram's method. While these will grow easily on ordinary media this method of differentiation is seldom used since the point of importance is that the pseudo-gonococcus never occurs in large numbers. For the recognition of the gonococcus it is necessary to find groups or clumps of these organisms in a smear, not only one or two on a side.

ACUTE ANTERIOR URETHRITIS.—The discharge from a case of acute urethritis, very early in the case (that is, for the first few hours), is scanty, of the color of water and milk, or starch solution, and consists chiefly of serum and epithelial cells, very few leucocytes, often some red blood cells, and a few gonococci, chiefly extracellular. After a few hours, however, the discharge becomes abundant, yellow in color, creamy in consistency, and composed of almost pure pus, and often some blood. Gonococci are easily found. During the first very few days it is interesting that the gonococcus is the only organism found. After this the ordinary rich urethral flora returns.

In an untreated case, which may recover spontaneously after from four to six weeks, the discharge becomes again starchy, more and more scanty, and consists of abundant mucus, and fewer pus cells. The epithelial cells are more numerous and the gonococci become fewer and fewer. It may take repeated searches to find the organism. Finally there is a discharge of almost pure mucus which often greatly distresses the patient. It contains no pus or gonococci. In case the disease had not extended beyond the anterior urethra the patient is now well. But only too often the infection travels from the anterior to the posterior urethra, and to the adjacent structures. Also secondary infections by pyogenic organisms are common, and these modify the exudates much.

In posterior urethritis the discharge is often profuse, but, restrained by the compressor urethræ muscle, it all flows back into the bladder, and is voided with the urine. The discharge from the anterior urethra at this time may be very scanty. If the patient pass his urine in two portions, the discharge from both posterior and anterior urethra will be washed out with the first portion of urine, and if the amount of exudate from the posterior portion be small, the second specimen of urine may be clear. But usually the second glass also will contain pus, since the exudate has been flowing back into the bladder, yet it should not contain as much as the first glass. If the anterior urethra is well irrigated with boric acid solution and then the patient voids into two glasses, the presence of pus in the first will indicate a posterior urethritis. The best time to try this test is with the first voiding in the morning.

The sequelæ of posterior urethritis are prostatitis, vesiculitis, epididymitis, and cystitis. In very acute posterior urethritis the frequent and excessively painful micturitions are very distressing symptoms. There is often a terminal hæmaturia. The whole urine may be bloody from the blood which constantly flows back into the bladder, but at the end of micturition a few drops or more of pure blood often flow from the urethra.

If a chronic urethritis follows, the discharge may be continuous

and fairly profuse or very scanty. In the latter case there is perhaps only enough to glue together the lips of the urethral orifice, or a little glairy fluid consisting of shreds of mucus enclosing a few pus, but more epithelial, cells. This discharge should be carefully distinguished from the glairy discharge following an acute urethritis. Smears should be carefully studied for pus cells and gonococci. This exudate is washed out of the urethra as *Tripperfäden* (see page 283). It is very difficult to demonstrate the gonococcus in them.

These "clap threads" when long, translucent, and branching are made up mainly of mucous which is "rolled up" from folds in the urethral mucosa; when short, thick, tack-shaped and sinking quickly to the bottom of the glass, they contain considerable pus and are supposed to come from the urethral crypts. Some of the shreds from the posterior urethra are short, slender, delicate, and comma-shaped. These are from the prostatic excretory ducts (*Fürbinger's books*). These shreds should be carefully examined for gonococci.

When abundant, the discharge may be a thick pus, in which case pyogenic organisms may also be present but this is not always the case, or muco-pus, or almost pure mucus. The flow is very intermittent.

If the patient voids into three glasses immediately after irrigating the anterior urethra and shreds are found in any of the glasses there is surely a chronic posterior urethritis. In these cases the whole volume of urine is often slightly cloudy, since the exudate is constantly flowing back into the bladder.

In women an acute anterior urethritis is of briefer duration and less apt to become chronic than in men. (Many deny this last point.) Next to the urethra, the cervix is the most common focus of infection. The discharge is at first slimy and blood-stained, and later a milky pus. In this location, especially, is the infection apt to become latent and chronic, and the only sign a viscid catarrhal mucous discharge.

The discharge in cases of vulvitis and vaginitis is often a profuse and very fetid pus, since so often there is a mixed infection.

**NON-SPECIFIC URETHRITIS.**—An urethritis due not to the gonococcus but to various other organisms, the colon bacillus, the diphtheria bacillus, streptococci, staphylococci, etc., does occur.

Smears from the exudate will show the presence of these organisms in great numbers, but it is well to remember the frequency with which secondary infections complicate gonorrhœa and the difficulty one often has to find the gonococcus in gonorrhœal cases.

In these non-specific cases the exudate is pus, but the discharge is not profuse, the case is mild and responds readily to treatment.

**BACTERIORRHŒA.**—This name is given to the condition charac-



terized by a discharge from the urethra of a thin opaque fluid which microscopically consists almost entirely of mucus and saprophytic bacteria of all varieties. No pus cells are present in the discharge. There are no other symptoms. The condition clears up quickly under treatment.

PROSTATITIS.—Prostatitis may be due to the extension of an infection from the urethra or from the rectum, or an infection by organisms from the blood stream.

The diagnosis of *acute prostatitis* is more a matter of physical than urinary examination. In *chronic prostatitis*\* the diagnosis is made by physical examination but also by the examination of the prostatic fluid which one obtains by massaging the prostate gland after the urethra has been well irrigated.

The normal prostatic fluid has been described on page 312. The fluid in a case of chronic prostatitis may contain some or all of the normal constituents in varying amounts, or none of these. No diagnostic or prognostic value can as yet be ascribed to the presence or amounts of these normal constituents. The abnormal element of greatest importance is pus. In some cases at times, and especially at the first examination, no pus cells will be found, and later many. The amount of pus bears a fairly direct relation to the extent of prostatic involvement. Red cells are sometimes abundant. Spermatozoa, active or immobile, are found in varying numbers.

When there is so much prostatic fluid that a discharge follows urination or defecation the condition is called "prostatorrhœa" (also called "spermatorrhœa"). This usually indicates also a mild prostatitis.

The prostatic fluid is a thin, bluish skim-milk-like fluid, which in prostatitis is modified by the presence of pus. In chronic prostatitis the fluid is always alkaline to litmus.

The fluid which can be expressed from the seminal vesicles is thick and gelatinous, resembling boiled tapioca or sago. This fluid sinks in the urine. Its chief constituents are "mucin globules," large unformed masses resembling large non-nucleated epithelial cells (Fig. 60, *b*), spermatozoa (Fig. 61 represents a large mucin globule full of spermatozoa), pigmented epithelial cells, and small finely and coarsely granular non-nucleated epithelial cells.

Dr. Young† has emphasized the value of the *seven-glass test* in the differential diagnosis of chronic inflammatory lesions of the urethra, prostate, seminal vesicles, and bladder. The patient compresses the urethra far back at the root of the penis (at the suspensory

\* For complete description see Young, Johns Hopkins Hosp. Reports, 1906, vol. xiii, page 302.

† Johns Hopkins Hosp. Rep., 1906, vol. xiii., page 1.

ligament) while the anterior urethra is irrigated by means of a long irrigating tube. The fluid is caught in two glasses. The first, I<sup>1</sup>, will contain the shreds, if any are present, the second, I<sup>2</sup>, should be perfectly clear. The patient's fingers are then removed and the tube carried back as far as the deeper part of the bulbous urethra. The washing is again caught in two glasses. The first, I<sup>3</sup>, will contain the shreds from the bulbous urethra (if any are there), the second, I<sup>4</sup>, should be clear. The urine is then voided into three glasses, I<sup>5</sup>, I<sup>6</sup>, I<sup>7</sup>.

I<sup>5</sup>, I<sup>6</sup> and I<sup>7</sup> all will contain bladder urine, and mixed uniformly in each the exudate of posterior urethritis which has flowed back into the bladder between voidings. In addition to this

I<sup>5</sup> will contain the exudate in the posterior urethra which will usually be washed clean by the flow of urine,

I<sup>6</sup> may contain the last traces from the posterior urethra, and

I<sup>7</sup> will contain also urine from the most dependent portions of the bladder, also the contents of the prostatic and ejaculatory ducts which often do not discharge the exudate collecting in them until the muscular contraction made at the end of micturition forces out the plugs of thickened exudate which occlude their mouths (Fürbinger's hooks).

#### BACTERIOLOGY OF THE EXTERNAL GENITALIA

**BACILLUS ULCERIS CANCROSI** (Ducrey's Bacillus).—This organism is now recognized as the cause of soft chancre. It is found in smears of the purulent discharge from these sores, but always mixed with a host of other organisms. (Sections of the tissue show it in pure culture.)

This bacillus is a small oval rod about  $1.5\ \mu$  long and  $0.5\ \mu$  thick, which stains readily in all bacterial stains, but decolorizes very easily. It is a very poor grower indeed, but some claim it can be cultivated on blood agar.

**TREPANOMA PALLIDA, SPIROCHÆTE PALLIDUM**.—This organism (see Fig. 54d) is a spirochæte, the average length of which is from 4 to  $10\ \mu$  (although some are even  $20\ \mu$  long), and thickness  $0.5\ \mu$  or less. It is tightly twisted like a cork-screw in 3 to 26 regular curves, and the fineness of the curves is a characteristic feature. It is pointed at each end. It is a flagellated organism, but shows only a twisting, rotating, or bending motion. They are usually found singly, although sometimes several are tangled.

To obtain this organism from a chancre, the sore is first well cleaned with soap and water, rinsed, and dried. This removes the majority of *Spirochæte refringens*. The chancre is then lightly curetted, the blood wiped off, and the sore squeezed until a drop of blood stained serum exudes. A thin smear preparation is then made.

Enlarged glands may be examined by aspirating a drop of serum from them with a hypodermic syringe. Smears may be made from the skin rashes by removing the superficial layer of epidermis and squeezing a minute drop of serum from the papule. In the case of the blood, 1 cc. of blood is mixed with 10 cc. of 0.3 per cent. acetic acid, centrifugalized, and thin smears made from the sediment.

This organism stains very badly, so great care must be taken with the technic. The smear is fixed by simple air drying, or by holding it for a few seconds over the mouth of a bottle containing crystals of osmic acid.

One of the best stains is Giemsa's azur-eosin mixture:

Eosin solution (2.5 cc. of 1 per cent. eosin solution in 500 of water), 12 parts.

Azur No. I (1:1000 in water), 3 parts.

Azur No. II (0.8:1000 solution in water), 3 parts.

This stain must be used when freshly made up. The specimen is left in it for twenty-four hours.



FIG. 54d.—On the left *Trepanoma pallida* (*Spirochæte pallidum*). A smear from a chancre. On the right *Spirochæte refringens*. A smear from a chancroid.

The Giemsa mixture most in use is:

Azur II, eosin, 3 gm.

Azur II, 0.8 gm.

Glycerin (Merck C. P.), 250 gm.

Methyl alcohol (Kahlbaum I), 250 gm.

(It is better to buy stains of this character than to make them.) To use this stain the specimen is dried in the air and then fixed for one hour in absolute alcohol and stained for twenty-four hours in a fresh dilution of the stain (1 drop of the above mixture to 1 cc. of distilled water). In this stain the organism takes a delicate violet purple color, and the nuclei of the leucocytes a deep blackish red. (The latter is the criterion for a smear well stained enough to justify the long search necessary to find the organisms.)

MacNeal's stain is composed as follows:

Methylene blue (crude), 0.25.  
Methylene blue (medically pure), 0.10.  
Eosin (yellowish), 0.20.  
Methyl alcohol (pure), 100.

The specimen is heated on a cover glass in this stain for forty-five seconds. It is then moved about in sodium carbonate (1:20,000) solution for one or two minutes, washed in water and examined. The organism is stained a delicate blue or even black.

The India-ink method of Burri has the advantages of rapidity and simplicity. One loopful of serum, squeezed from the lesion, is mixed with one loopful of India ink (the best is the Chin-Chin liquid pearl ink manufactured by Gunther Wagner, but other kinds give fair results) on a perfectly clean glass slide, and the mixture is immediately spread with the edge of the other slide. The film dries without heat in half a minute. Without further fixation or mounting, the specimen is at once studied under the oil-immersion lens. The whole field is seen to be of a homogeneous brown or black color, with the spirochæte, blood-cells, etc., shining through as colorless refractive bodies. *Spirochæta pallidum* can easily be distinguished from other spirochæte in these specimens. Cohn \* highly recommends this method for routine work.

**SPIROCHÆTE REFRINGENS** (see Fig. 54d).—This organism is often found with *Spirochæta pallidum*. It is a common parasite, occurring in great numbers in many ulcerative lesions. It is larger, thicker, more refractile, its spirals are broader and more wavy, its ends are blunter, it occurs in greater numbers in a smear, and it stains more easily than *Spirochæta pallidum*.

On the skin about the genitalia is an abundant flora of organisms: streptococci, staphylococci (especially *Staphylococcus albus*), *Bacillus coli communis*, *Bacillus pyocyaneus*, *Bacillus lactis aërogenes*, *Bacillus aërogenes capsulatus*, and various strains of smegma bacilli.

**VEGETABLE PARASITES.**—See bacteriology of the urine, page 289.

**Yeasts.**—Ordinary yeast cells, saprophytes from the air or surface of the body, are sometimes found in the urine in cases of diabetes. If they gain entrance to the bladder they may ferment the sugar before the urine is voided and give rise to "pneumaturia." To cultivate them the urine should be kept acid with acetic acid. In cases of systemic blastomycosis the organism has been found in the urine.

**MOULDS** may perhaps sometimes occur in the urine when voided, but the spores accumulate on standing. In a recent case of pyelitis, the

\* Interstate Medical Journal, January, 1911, vol. xviii, p. 26.

pelvis of whose kidney had been repeatedly irrigated through ureteral catheters, on one occasion the urine which was obtained was very bloody, and in it were found mycelial masses of some organism which would not grow on media. After irrigating the pelvis out well no more appeared. Spores may have been introduced at a previous catheterization.

SARCINÆ smaller than those found in the gastric cases may also be found.

Among the **Animal Parasites** which either in part or whole may be demonstrated in the urine are the hooklets, daughter cysts (even several hundred in a case), and fragments of membrane of ECHINO-

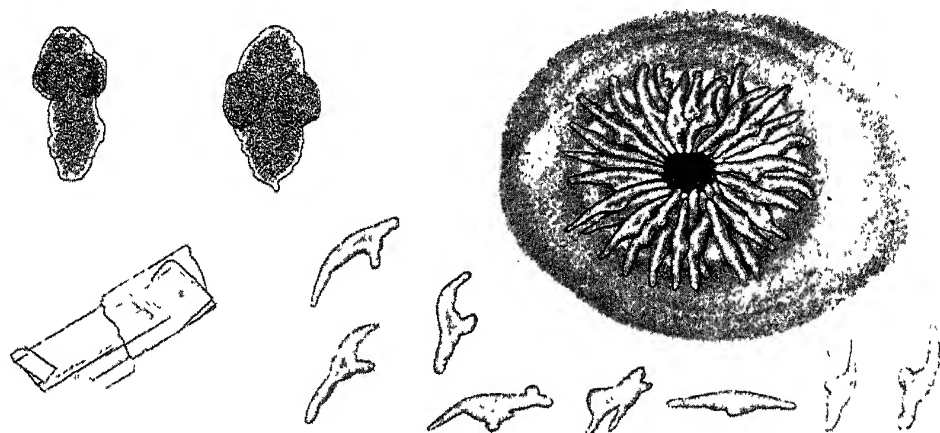


FIG. 55—Sediment from echinococcus cyst. Above and to the left are two degenerated scolices ( $\times$  about 60); to the right is the head of a scolex ( $\times$  400); below are hooklets of unusual shapes and a small mass of cholesterol crystals.  $\times$  400.

COCCUS CYSTS. There are no urinary symptoms of hydatid disease of the kidney unless there be a catarrhal pyelitis or unless the cyst empties into the urinary tract. If the latter be the case the urine will appear watery, soapy, or bloody. Embryos of filaria are found in tropical hæmatochyluria. (See page 670.)

FLAGELLATES belonging to the cercomonas or the trichomonas class occur. There is a dispute whether these are present before voiding or are a later contamination of the urine.

EUSTRONGYLUS GIGAS.—A few cases are reported, but in many they were mistaken diagnoses. In one case of chyluria these eggs were found.<sup>160</sup> (See Fig. 58a.)

SCHISTOSOMUM HÆMATOBIUM (BILHARZ).—This trematode worm (see Fig. 57), so common in Africa, especially in Egypt and

<sup>160</sup> Stuertz, Deutsches Arch. f. klin. Med., 1903, vol. lxxviii, p. 586.

the Transvaal, has been found only six times in this country<sup>161</sup> and only twice in Porto Rico (Martinez).

The male measures from 12 to 14 mm. in length, is flat, but so folded that it appears cylindrical, and encloses a gynecophoric canal which receives the female. The female is 20 mm. long and filiform.

The adults live in the portal vein and its branches as well as in the other veins of the abdomen and pelvis, especially those of the bladder, the pelvis of the kidney, and the rectum. The eggs (Figs. 92a, 92b) are large, from 120 to 190 microns long and from 50 to 73 microns wide, and fusiform, have no operculum, and have a spine which may be terminal or lateral (*Schistosomum mansoni*?).

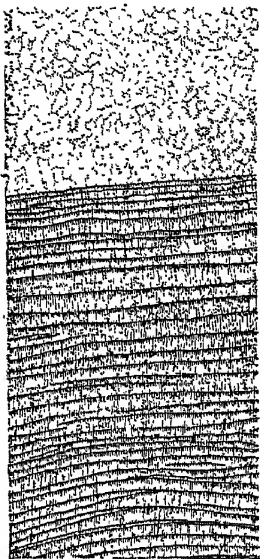


FIG. 56.—A small fragment of echinococcus cyst-wall on cross fracture, showing transverse striation and pectination.  $\times 50$ .

The urinary symptoms of the infection are catarrh of the bladder and hemorrhages ("Egyptian hæmaturia"). At first bloody flecks are passed at the end of micturition, but later the hemorrhages may be profuse. The symptoms are caused by the eggs which the female deposits in the mucous membrane. They may get into the lumen of the bladder and be passed with the urine, but many of them, especially those with a lateral spine, remain in the bladder, and these often form the nuclei of calculi. The egg contains a miracidium which is completely ciliated. The shell splits when the urine is diluted with water. What the intermediary host is, and how human infection occurs, are not known.

NEMATODE WORMS other than filaria are sometimes found in the urine, some of which may be *Anguillula aceti*, or "vinegar eel." Stiles reports one case of infection of the bladder with this worm. Other cases may be due to contamination from the bottle in which the urine is collected.<sup>162</sup> These worms resemble closely *Strongyloides intestinalis*, except that these worms (*A. aceti*) are slightly longer. (Males, 1.2 mm. long, 0.033 mm. wide; females, 1.9 mm. long, 0.06 mm. wide; embryos, 0.25 to 0.3 mm. by 0.015 mm.)

The student should always be able to recognize the various plant contaminations which occur in water and hence in vessels rinsed out with this water; that is, he should be able to say that they are plants and of no significance. A few of the most common we give in Fig. 58.

**Prostatic Fluid.**—This fluid is best obtained by "milking" the prostate through the rectum. The urethra is first well washed, then the

<sup>161</sup> See O'Neil, Boston Med. and Surg. Jour., October 27, 1904, vol. cli, p. 453. See also, Dr. Daywalt's letter in Dr. Arnold's paper, Southern Practitioner, 1906, vol. xxviii, p. 13.

<sup>162</sup> Billings and Miller, American Medicine, May 31, 1902.

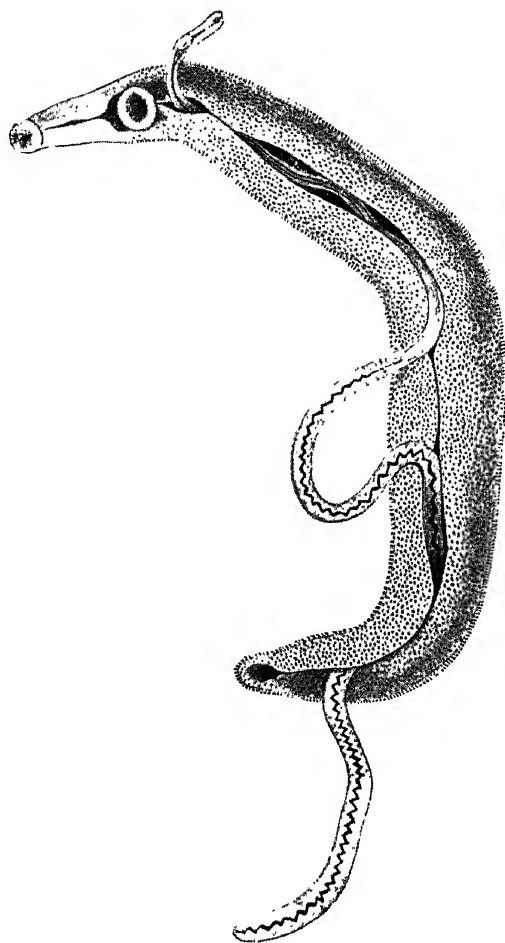


FIG. 57.—*Schistosomum hæmatobium*, adult worms. (Copied from Braun.)

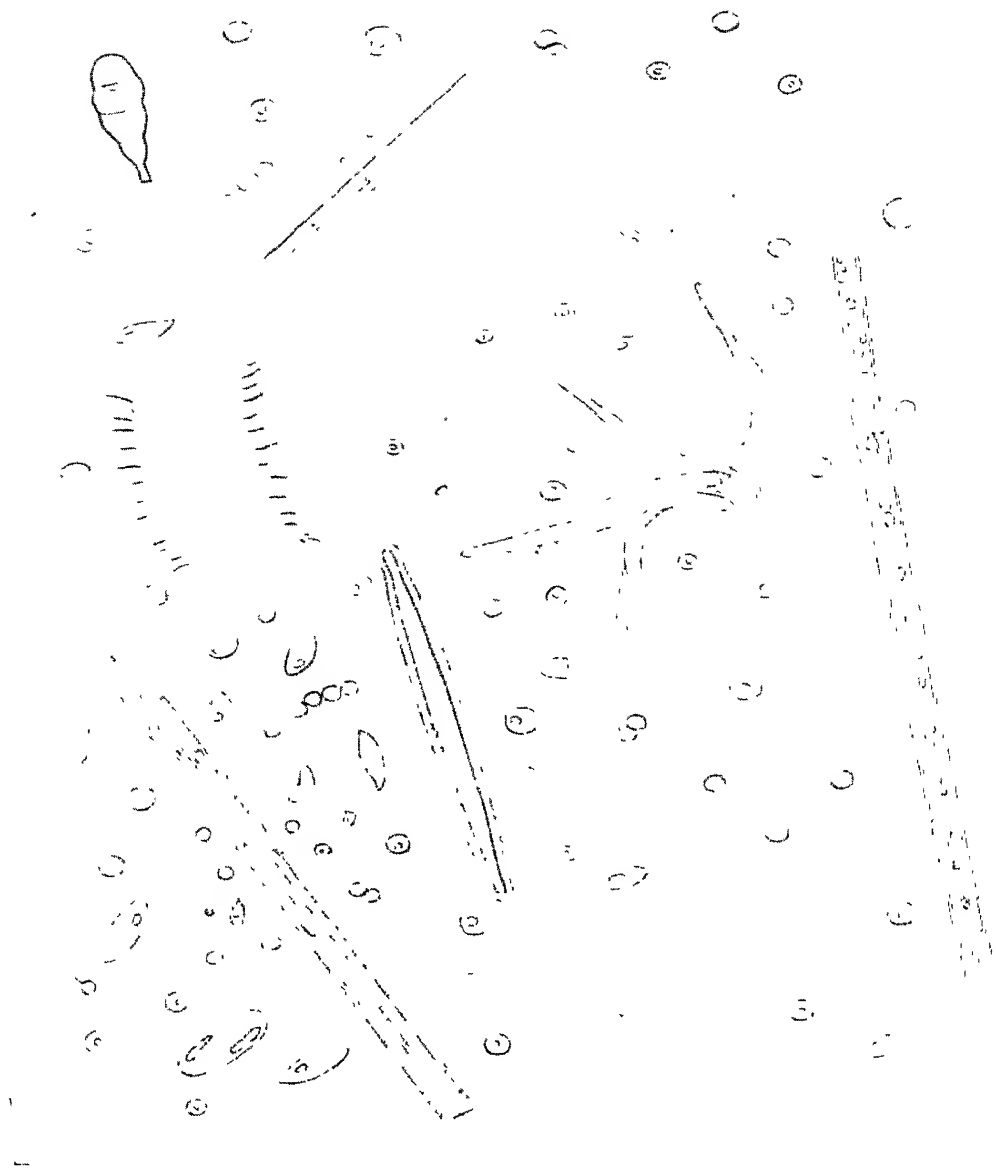


FIG. 58.—Protophytes and other low forms of life often found in tap water.  $\times 400$ .



FIG. 58a.—Eggs of *Eustrongylus gigas*.  $\times 400$ .



fluid expressed and collected. The amount varies greatly, from none to even 5 cc. of normal fluid at one milking. It is of a grayish-white, yellow, or greenish color, with a milky turbidity due to lecithin globules, and of a characteristic odor. It is slightly viscid, tenacious, of light specific gravity since the solids are but 1 to 2 per cent., faintly alkaline as a rule, although it is acid to some reagents; its reaction varies very much, and is as yet a very uncertain quality.

One examines first fresh for motile spermatozoa, then adds a drop of acetic acid to bring out the cells more clearly, and examines for pus-cells. (Fig. 59, b.)

Microscopically, the most striking objects are the great numbers of *lecithin globules* (Fig. 59, a), which give it its milky appearance. These vary in size from those minute to others even half the size of a

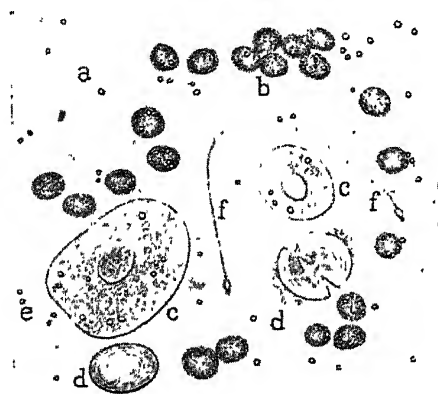


FIG. 59.—Prostatic fluid. ( $\times 400$ .) a, lecithin globules; b, pus-cells; c, epithelial cells; d corpora amylacea; e, free granules from epithelial cells; f, spermatozoa.

red blood-cell. They are not very refractive, and can be easily distinguished from fat. Their only significance is that an increase is a good sign in cases of chronic prostatitis. *Corpora amylacea* (Fig. 59, d; 60, c) are often found, especially in advanced life, in which case they also appear in the urine. They are laminated, with a finely granular centre, often a nucleus. Of their composition nothing is known except that they stain blue with iodine; they have no significance. *Epithelial cells* of various kinds are present. Some are large, polygonal, single or in groups, and of very varying size (see Figs. 59, c, 60, a). Other cells, the so-called granular cells (Fig. 60, e), also of varying size, are simple masses of granules resembling fat, some of which resemble colostrum corpuscles. These break down, hence the refractive globules seen free in the fluid (Fig. 59, e). Some granules resemble myelin (Fig. 60, d). Columnar epithelial cells are sometimes present. In addition to these are large clear cells of very varying size, with or without nucleus (see Fig. 60, b), which are supposed also to be derived from the seminal vesicles. There are normally no pus-cells, nor any red blood-corpuscles.

*Spermatozoa* (Fig. 59, f) are usually present. (For a description of these, see University of Pennsylvania Med. Bull., No. 3, 1902.)

These should be examined fresh, to make sure of their motility; to study them more carefully smears are made, dried in the air, heated to 120°, and cooled slowly. The fluid should first be diluted with water even of twenty volumes in case much proteid be present. They are best stained in iron hæmatoxylin. The specimen fixed by heat is placed in 2 per cent. iron alum solution for from two to four hours, washed in water, then in 1 per cent. hæmatoxylin for twelve hours. They are decolorized with 1 per cent. iron alum carefully, and counterstained with saturated aqueous solution of eosin from one to three minutes, then dried and mounted. Many of them are abnormal in shape, some with two heads and with even three tails. These monsters seem never to move.

One seldom tries to determine more than their presence and motility; if motile, one is confident that they are functionally normal; if absent or non-motile, no conclusions are justified.

In acute or chronic prostatitis many leucocytes are present, and lecithin is diminished.

*Spermin crystals* resemble somewhat the Charcot-Leyden crystals, being colorless, transparent needles or whetstones, but are often imperfectly crystallized. To demonstrate these the semen is allowed to stand. To the prostatic fluid, however, must be added one drop of 1 per cent. ammonium phosphate and the specimen allowed to dry under a cover-glass for two hours.

The PROSTATIC CASTS which are said by some to resemble the urine casts markedly must certainly be rare, since we have asked those who have examined many hundreds of specimens, and they have never seen one.

TRIPPERFÄDEN.—These threads, which are seen grossly in the urine in cases of chronic gonorrhœa, are present as narrow delicate transparent mucous flocculi, which microscopically contain mucus and a few epithelial and still fewer pus-cells; this form is present in very chronic cases; or shorter, firmer bands, in which the cellular elements, especially the pus-cells, predominate. They settle at once to the bottom of the glass, but are evident on agitating the urine, upon which the threads rise from the bottom. In an old urine they may be difficult to demonstrate, since they have coalesced.

Also short coma-like flocculi are seen which arise from the excretory ducts of various glands and follicles, and mean an intense involvement of the urethral glands. Those in the second glass are from the glands in the prostate, and are signs of chronic prostatitis. They consist of superimposed layers of cylindrical epithelium.

PROSTATIC PLUGS are sometimes found which are large cylindrical masses of mucus. These are found in mild inflammations of the prostatic ducts. Other mucous masses are found full of spermatozoa (Fig. 61).

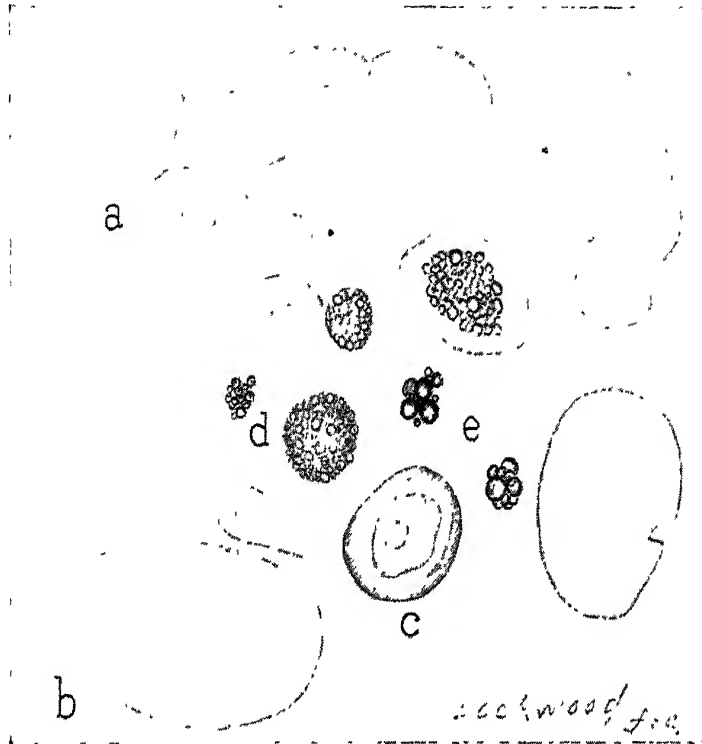


FIG. 60.—Prostatic fluid, *a*, epithelial cells, *b*, clear epithelial cells, from seminal vesicles(?), *c*, corpus amylaceum; *d*, “granular cells” with droplets resembling myelin, *e*, “granular cells” with fat droplets.

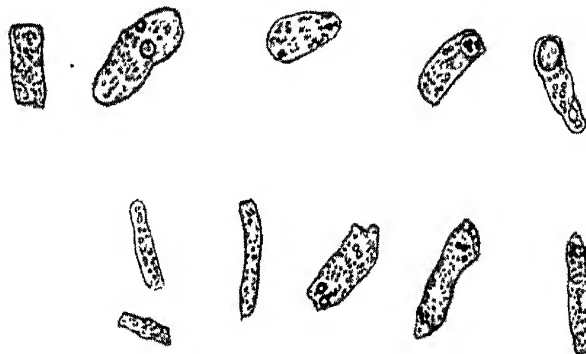


FIG. 60a.—Cells which resemble casts found in fluid massaged from a prostate, the seat of chronic prostatitis. (Kindness of Dr. George Walker, of Baltimore, who will later publish this and similar cases )



## DISEASES OF THE KIDNEYS

**Albuminuria.**—In order to get a general idea of the occurrence of albuminuria, the conditions in which it occurred most commonly, and, if possible, obtain some clue for further investigation in this important subject, we have abstracted the histories of 3631 hospital cases, taking them in order of admission to the hospital without reference to their diagnosis. Only such histories were abstracted the urine examinations in which appeared to us perfectly satisfactory.

It very soon became evident that the age line, that is, the occurrence of albumin in the various decades, is to be first determined. So important is this that the effect of any given agent or disease upon

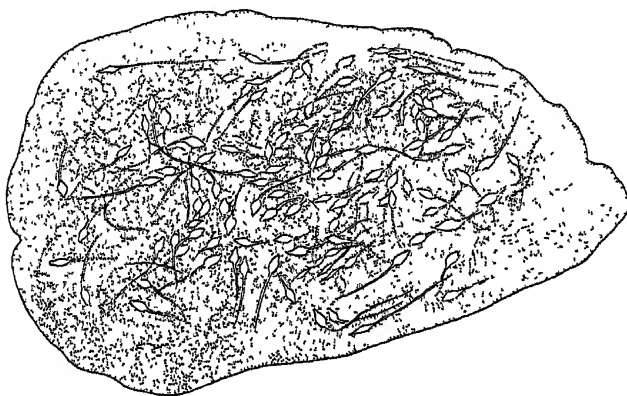


FIG. 61.—Mass of mucus filled with spermatozoa from urine catheterized at death.  $\times 400$ .

the kidneys can only be rightly determined in case the age be taken into consideration.

We have divided the cases into three groups,—those in which the urine was throughout the stay in the hospital albumin-free; those in which the albumin was present for a time but disappeared while under examination; and those in which albumin was present at each examination. As the age epochs we have chosen from one to fifteen, sixteen to twenty-five, twenty-six to thirty-five, thirty-six to forty-five, and so on through the epochs. The reason for choosing these figures is that the ages of fifteen and twenty-five are more truly transition points in a person's life than ten and twenty. Not only should the urine be studied by decades, but also the sexes should be studied separately for certain decades at least. On the whole, the sex has much less influence than one would expect. These figures will be published in full later. The neurasthenics may be taken as representing a group of normal men, for this diagnosis represents exclusion of other conditions. Of the men, the percentages with albumin-free urine were: one to fifteen years, 100 per cent.; sixteen to twenty-five, 87 per cent.; twenty-six to

thirty-five, 99 per cent.; thirty-six to forty-five, 90 per cent.; forty-six to fifty-five, 84 per cent.; fifty-six to sixty-five, 70 per cent.; sixty-six and over, 66 per cent. The drop at the period of adolescence is very interesting (see page 232).

Of the FEVERS, typhoid after the twenty-fifth year is accompanied by a transitory albuminuria (febrile) in 30 per cent. of the cases, and a persistent albuminuria in about 30 per cent. One would expect this, since the fever is so continued and bacilluria is common (about one-third of all cases). Yet as a disease in the past history, typhoid fever strangely enough seems to have the least effect on the kidneys, notwithstanding that it has a deleterious influence on the peripheral blood-vessels.

Malaria of the tertian and quartan types has little effect on the kidney, æstivo-autumnal much. Pneumonia has the highest percentage of transitory albuminuria of all the fevers we studied (but about 25 per cent. of the cases are albumin-free), but almost no permanent effect. Pulmonary tuberculosis and acute articular rheumatism cause little febrile albuminuria. Of the AFEBRILE DISEASES, the neurasthenics are the best off; the arteriosclerotics the worst. In fact, this disease, arteriosclerosis, seems the one dominating element among the causes of albuminuria.

The relation between the anatomical lesions and urinary findings was studied.

Cases with marked CLOUDY SWELLING at autopsy, but no other lesion, as a rule have had an albuminuria, usually slight, extending for two or three weeks before death. But in a few cases there was none even shortly before death. Casts are also present with albumin, usually hyaline, but also waxy, epithelial, and blood.

FATTY KIDNEYS (no other microscopical changes) are seen in various diseases, and have been divided into those with fatty infiltration and fatty degeneration; the former occurring in diabetes mellitus, pregnancy, etc., the latter in various infectious diseases, cachexias, anæmias, and following various poisons.

The amount of urine is usually normal, although in some severe cases, decreased; albumin is present in various amounts, a trace or much, and a relatively large number of casts, hyaline, granular, fatty, and epithelial; with few or many red corpuscles.

All of our cases examined had excreted albuminous urine before death, but in no case exceeding two weeks. Hyaline and granular casts were present.

The urine of kidneys with CHRONIC PASSIVE CONGESTION is at first scanty, dark in color, very acid, the specific gravity between 1025 and 1030. The urate sediment is often abundant. Urobilin and uro-

erythrin are increased and sometimes bilirubin is present. Sooner or later albumin appears in traces, later in good amount, even 0.1 per cent., and in one of our cases 0.6 per cent. Casts are present, chiefly hyaline, rarely granular. Yet here also on some days casts may be present in great numbers, hyaline, waxy, epithelial, and fatty. A very few leucocytes may be found and still fewer red cells. The points of importance in this urine are the small amount of albumin, the large urate sediment, the absence of renal epithelium, the scarcity of granular casts and leucocytes. The diagnosis of nephritis had been made in over half the cases.

**Acute Nephritis.**—Acute nephritis has been divided into several groups, chiefly from the stand-point of pathology, but for the clinician a division is very difficult. Senator separates the tubular nephritis or acute parenchymatous from the acute diffuse. In the *acute parenchymatous* the tubules especially are involved, and the glomeruli but little or not at all. The clinical symptoms are slight if any. The urine shows only a slight febrile albuminuria, a diminished amount of urine of rather high specific gravity, and few or no casts. From this form are all gradations to the acute diffuse nephritis. The urine contains often a heavy sediment, chiefly of renal epithelium, and hence the name “nephritis desquamativa.” The epithelial cells may be single or in casts. Hyaline casts, few or many, are present. Crystals of uric acid and calcium oxalate are often present, red blood-corpuscles and hæmoglobin in granular casts or masses. The leucocytes are usually few in number. The albumin is nearly always slight in amount, in remarkable contrast to the amount of sediment, and some claim that nearly all of it is nucleo-albumin from the cells.

In the acute diffuse nephritis, a good illustration of which is that following scarlet fever, the clinical symptoms are much more severe. The urine is diminished in amount, there may, indeed, be anuria for the first twenty-four hours. From 50 to 100 cc. for the first day or so is not uncommon, but later from 200 to 500; specific gravity high, even 1030. Toward death there may be a diminished or an increased amount voided. The specific gravity was normal as a rule, 1015 to 1017, but in some cases high, from 1023 to 1025 (when the urine was from 300 to 600 cc.). It is usually of a dark color and cloudy. In very mild cases, however, it may appear normal. Blood is nearly always present. When slight in amount it imparts to the urine a slight smoky tinge, which may be recognized grossly. When larger in amount the urine may have a reddish-brown or a brownish or even a chocolate color, according to the proportion that is present between hæmoglobin and methæmoglobin. Albumin is an almost constant feature, and yet in some fatal cases there may be but traces, and these only on a few days, and alternating with periods of none, even till

death. Serum albumin and serum globulin are present. If many cells are present in the sediment a certain amount of true nucleo-albumin may be expected. Albumose is also present, and in some cases is the only proteid found. The reason for this is not known. It may explain, however, the cases described as albumin-free, the examiner using only a heat test, which did not precipitate the albumose. As a rule, the albumin is not above 1 per cent., and the globulin percentage is relatively high. In the sediment may always be found red blood-cells, mononuclear cells, few polynuclear leucocytes, and epithelial cells from the urinary tubules, which are present singly or in masses, and often very fatty. Among the crystals uric acid and calcium oxalate are found, and hæmoglobin either in amorphous granules or in casts. In the hemorrhagic form of the disease in our series the red blood-cells were evidently remarkably few in number. The leucocytes were very abundant in one case of acute nephritis with multiple abscesses. Casts are present in varying numbers, and may be of any form; epithelial, hyaline, and coarsely granular will predominate, blood and leucocyte casts may also be present. As a rule, the number runs parallel to the amount of albumin, yet it varies greatly from day to day, and on some days may be enormous. In one case of acute hemorrhagic nephritis, with areas of complete necrosis, the amount of albumin was slight but large numbers of casts, leucocyte and granular, were present. In one case of general septicæmia the albumin occurred in but traces on certain days and was absent on others, and yet blood, hyaline, and leucocyte casts were found.

During the course of an acute attack of nephritis the kidney shows every symptom of renal insufficiency. The nitrogen output is diminished, not due to the diet alone. The chlorides and the phosphates are diminished, and hence the molecular concentration is less than normal. The uric acid output is about normal, that of the xanthin bases is said to be increased. The ability of the kidney to form hippuric acid is diminished, and the glycosuria after phlorizin is either slight or absent. In mild cases and in severe ones as they improve the urine approaches normal. It is said that the albumin disappears last, but we believe the casts are often found later.

**Nephritis Hæmoglobinurica.**—In acute nephritis the amount of hæmoglobin in the urine may be much, the number of red blood-cells few or none. In certain cases this is the cause of the nephritis, in others a symptom. The former is true in cases with blood destruction due to poisons, burns, etc. During infectious diseases the hæmoglobinuria may be secondary, or both that and the nephritis due to the same cause. Such is found in typhoid fever, scarlet fever, malaria, Winckel's disease of the new-born, and other conditions. This form of nephritis differs from pure hæmoglobinuria by the greater amount of albumin



and the richness of the sediment in casts, renal epithelial cells, leucocytes, and uric acid crystals.

**Acute Nephritis of Cholera.**—This form is said to be a peculiar type of a pure parenchymatous, especially the tubular variety. The urine is diminished even to anuria for from five to seven days even. It is very rich in salts and may have a large urate sediment. Albumin is present in relatively larger amounts than in the other forms of parenchymatous nephritis. The urine is dark and cloudy, but rarely bloody. Hyaline and granular casts are present, also renal epithelium, red blood-cells, leucocytes, uric acid and calcium oxalate crystals. The urine is also characterized by its richness in the ethereal sulphates. Diacetic acid is often present and ammonia is increased. In one case after an anuria of fifteen days the person recovered. The condition of the urine improves much during the stage of reaction.

**The Nephritis syphilitica acuta præcox** is sometimes marked by the immense amount of albumin present, in one case (Hoffmann and Sal-kowski) 8.5 per cent. The urine coagulated solid. There was very little sediment, few casts, leucocytes, or blood-cells.

**Subacute Nephritis. Chronic Parenchymatous Nephritis. Chronic Diffuse, Non-Indurative Nephritis. Large White Kidney.**—This form of subacute nephritis, which may follow an acute attack or develop without this, is characterized by its subacute course, for it is usually fatal within two years, and by the extreme œdema and the effusions in the serous sacs. It occurs especially in young persons who work hard amid exposed, unhygienic surroundings. It may follow constitutional diseases, as tuberculosis, lues, or malaria; also the use of alcohol. The diagnosis is usually easy from the history.

The amount of urine is always diminished, the diminution varying as the œdema, and especially at death. At the height of the disease it varies from about 250 to 500 cc., as the case improves, however, it increases, and if the patient be encouraged to drink he may void from 5 to 6 litres of a very dilute urine. The amount also is increased as the œdema or the effusions begin to absorb. The specific gravity, varying inversely as the amount, is, as a rule, almost normal or slightly increased, in some cases reaching 1040. The reaction is faintly acid, but in some cases alkaline even on voiding, and in all cases it quickly becomes so. This makes a search for casts difficult. The color is from a pale greenish-yellow to a reddish or a reddish-brown, cloudy as a rule from the large amount of sediment, and foaming easily on shaking because of the amount of albumin that is present.

This is the form in which the albumin is very large in amount, both relatively and absolutely. The amount varies as the specific gravity, roughly, and seems to bear no relation to the œdema. It seldom

reaches 1 per cent., although for months it may vary from 0.4 to 0.8 per cent. In certain cases, however, it is greater. As the case changes to the chronic indurative form the amount of albumin becomes less and less. Cases of 2 per cent. are rare, and Bartels has reported a case which varied from 4 to 6 per cent. The albumin quotient varies much. Nucleo-albumin is present in small amounts, also the albumoses.

The urea is somewhat diminished, even when there is much dropsy. The uric acid varies somewhat less than the urea, and is excreted within normal limits. The ammonia is normal. There is a certain retention of chlorine and phosphoric acid.

The sediments are much the same as in acute nephritis, but it is more common to find coarsely granular, fatty, and waxy casts. Red blood-cells may practically always be found, in especially large numbers in the acute exacerbations. There is little difference between the urine of the white and the mottled kidneys except, perhaps, in the latter there are more red blood-corpuscles, leucocytes, and fatty cells.

Functionally the kidneys are somewhat insufficient, and yet in the severe cases they do their work fairly well. This is thought to be due to the fact that the disease attacks certain successive parts of the kidney, and that while one part is inflamed the other parts can compensate.

One would expect that a glomerular involvement would affect particularly the amount of albumin, the tubular involvement especially the number of casts, and while in general this may be true, clinically it is of little importance, since the two anatomical conditions are always present and variations between them but slight.

Except in young persons with a good past history, the diagnosis cannot be made without an autopsy, since an acute exacerbation of an unsuspected chronic case will show similar clinical features and very similar urine, yet at autopsy small contracted kidneys be found.

**Chronic Indurative Nephritis.**—A subdivision of this form is exceedingly difficult; in fact, the size and color of the kidney are almost the only criteria, since all its histological elements are affected in nearly every case by degenerative, inflammatory, or regenerative processes. A somewhat related form of nephritis, "senile atrophy," is of almost physiological occurrence in every elderly person, the kidney becoming old and therefore slightly sclerotic with the rest of the body. In fact, Dr. Osler emphasizes the point that in men above middle life the cortex is never perfectly normal—a few sclerotic glomeruli and a very slight increase of connective tissue may always be found. If the process, however, is simply sclerosis, there should be no urinary symptoms, and hence such cases are not suspected before the autopsy. As a result, however, of hard work, various diseases, gout, lues, and

certain poisons, lead and alcohol, inflammatory processes develop. The result in such a kidney is the degeneration of the epithelial elements, and inflammation resulting in new growth of connective tissue which may be general or focal, subcortical or periglomerular. The kidney becomes hard and firm, diminishes in size, and is finally but a remnant of an organ. In some cases we find contracted kidneys at autopsy which could not possibly have been recognized from the urine. These show that a nephritis limited to foci can heal. In all such cases, however, if it be the sclerotic process which predominates, there may be practically no urinary symptoms, and at autopsy kidneys of surprisingly small size may be found. Other cases are the result of a preceding acute or subacute nephritis.

The only classification which can be made, apart from the weight of the kidney and the thickness of its cortex, is in its color, and hence the division into the red and the white kidney, the red kidneys being the result particularly of arteriosclerosis as a primary factor. The red kidneys are large, firm, beefy, the sclerosis is considerable, and yet the size of the kidneys is seldom as much diminished as in the white form. In all cases it should be borne in mind that arteriosclerosis will be present, in some as the cause of the renal trouble, in others as the result. In a third group, both the renal and the arterial diseases are due to the same cause. The process may be local, one kidney affected more than the other.

**Chronic interstitial nephritis** in its advanced forms is marked by its very insidious onset. Its only symptom may be a slight albuminuria, and this may be absent for long periods. Later the albumin becomes permanent, casts appear, and later polyuria. The urine is increased slightly at first, but in a well-developed case from 2 to 3 litres are voided daily, and rarely even as high as 12 litres. On the other hand, it may at times sink to normal or even under. It is pale, clear, definitely acid, and of a specific gravity which is constantly between 1010 and 1005. This low specific gravity in a morning urine is always significant of this condition. The molecular concentration is diminished. The albumin seldom rises above 0.05 per cent., and usually is in mere traces. It is often absent in the morning voiding, and may indeed be present only after a day of unusual exercise or an especially hearty meal or some unusual excitement.

On the other hand, hyaline casts can usually be found on long centrifugalization. Red blood-cells are very common, sometimes hæmaturia occurs. There is often a desquamation of the epithelium cells of the tract, giving a cloudy urine resembling cystitis.

In the **arteriosclerotic kidneys** the urine contains albumin which occurs late and is often intermittent. Cases of the so-called "contracted kidneys with albumin-free urine" belong here, and yet in these

cases albumin is found more constantly and in larger amounts than in those of the preceding group. In the arteriosclerotic group of "small red kidneys" when the abnormal urinary findings disappear for a while it is often the casts which disappear first, leaving a pure albuminuria; and in the primary contracted group of "small white kidneys" the albumin often disappears first, leaving a pure cylindruria.

The urea is normal, the nitrogen normal, but the percentage of the various nitrogenous bodies may vary somewhat, in uræmia the ammonia rising at the expense of the urea. Uric acid is low and the xanthin bases are increased. The various tests for the functional activities of the kidneys, for instance the methylene blue and the KI test, sometimes indicate a pathological condition but more often do not. The sediment is scanty and difficult to find. The urine should be centrifugalized and search made over large amounts of urine. After a long search but one or two casts may be found. These are usually hyaline, although sometimes finely granular. Renal epithelium is sometimes found; often a few leucocytes, rarely a red cell, although many may be found after overexertion. Uric acid and calcium oxalate crystals are common.

During acute exacerbations of chronic nephritis the urine may closely resemble that of a more acute form.

For the diagnosis of chronic nephritis one should examine the morning and the evening urines separately, and that voided after a hearty meal or severe exercise. And yet the urine in this condition resembles that found in other conditions so nearly that the clinical history and the physical examination of the patient cannot be dispensed with. The urine alone resembles that of acute or the subacute nephritis during convalescence, that of waxy kidney, and the cyclic, or the so-called physiological albuminurias.

**Amyloid degeneration** may be superimposed upon any form of nephritis of which it really forms no part. When alone, the urine is said to be normal. In the majority of cases the condition could not be suspected from the urine, although given a case with history and physical signs indicating it, and the urinary changes may be well explained. Without the clinical features the urine would suggest, when concentrated, chronic passive congestion; when dilute, small contracted kidney. The classical description of the urine is that it is increased in amount, is pale, clear, faintly acid, of a low specific gravity, 1005 to 1012, that it contains abundant albumin with relatively much globulin, and very few casts. This picture of Traube, however, is rare. The albumin may occur in traces or fail, and the casts may be numerous. The casts are often fatty. Renal epithelium is rare, and red blood-corpuscles are extremely so.

**Uræmia.**—Uræmia may be considered the highest expression of renal insufficiency, but this is not all. Compensatory changes occur in nephritis, and the body may become tolerant to the toxins, whatever they may be. For this reason uræmia is more common in acute than in chronic nephritis, for in the latter the body has adapted itself to the condition. For these reasons, also, it is easily understood that the urine should show no evidence of an oncoming or present uræmia, for only functional activity can be tested and that but imperfectly.

But, on the other hand, it is of interest that in cases of anuria due to calculus or the removal of a single kidney, uræmia and death may not follow for from ten to fourteen days. This is good evidence that the retention of urinary constituents alone is not enough to explain the development of uræmia in a case of nephritis without demonstrable renal insufficiency.

We have abstracted the histories of 96 cases of nephritis with uræmia. Of the 54 cases in which the first symptom of the nephritis dated back less than six months, 42 died. Of the 35 cases with an old history of nephritis, 29 died. Senator states that the amount of urine is diminished or there is even anuria. This rarely fails, and yet very rarely there is polyuria at the onset. The total nitrogen is increased often, but the greatest interest for us at this point is the value in such cases of urea determinations made by the Doremus method. We will not here discuss the method itself. It has been used almost daily as a routine in all severe cases of nephritis. In only eight of these cases of uræmia was urea determination of possible value indicating either its onset by a drop or the improvement of the case by a rise. These may follow, be coincident with, or may precede the change in the symptoms. As the latter was true in but two cases, it is seen how very rarely it is that the oncoming uræmia can be foretold by the urea determination. Of course, in nephritis, as in all conditions, the amount of nitrogen eliminated depends chiefly on the amount of protein ingested, and since cases with impending uræmia eat poorly, those in uræmia none at all, and those improving eat better, the nitrogen curve will depend much on the food. But the question is, Does the nitrogen curve rise or fall? If we can judge from the urea it falls no lower in uræmia than in cases of nephritis without it.

There were 13 cases during uræmia with the urea under 1 per cent. We have dealt with percentages since it is almost impossible to get a full twenty-four hours' amount in such cases. Of these 13 cases, the average was 0.74 per cent.; in one case no urea could be determined. Omitting extremes, in 9 cases the urea varied from 0.6 to 0.9 per cent., an average of 0.8 per cent.

In 21 cases in which the urea was no help at all it varied at the onset of the convulsions from 0.9 to 3 per cent., an average of 1.4 and

a mean of 1.4 per cent. The rise in urea in cases with improvement is striking.

Of 123 cases of nephritis without uræmia and without much polyuria, in 33 per cent. the urea was at times at or under 1 per cent. In eighteen fatal cases the urea at death varied from 0.3 to 3 per cent., a mean of 1.2 per cent., an average of 1.4 per cent.; that is, exactly the same as in the above uræmic cases.

Uræmia may occur in any variety of nephritis, and with albumin present from a trace to a large amount. It is interesting how little this striking clinical crisis is evidenced in the urine.

One group of ten cases was interesting, since if improvement in condition be indicated by a diminution in albumin and casts with the same output of water, then uræmia may improve a case for a while at least. This of course may not always even suggest improvement, since in two the urea diminished with the albumin and casts, but in four it increased.

In eight cases of terminal uræmia the albumin increased in all. In one case of uræmia with a trace of albumin on the day before and the day following, on the day of the convulsions there was a large amount with a great number of casts.

In *eclampsia* the urinary features are similar to uræmia.

The temporary and extreme albuminuria occurring in *eclampsia* is quite striking. In a recent case in the maternity ward the albumin at 10 A.M., March 6, contained 0.653 per cent. albumin. The woman was then in the first stage of labor; and then convulsions began. The urine between 10 A.M. and 5 P.M. of that day contained 1.23 per cent.; between 5 P.M. and 9 P.M. 0.19 per cent.; at midnight, 0.075 per cent.; 3 A.M., 0.025 per cent.; and March 7, merely a trace. That is, in about twelve hours the output had decreased from 1.20 per cent. to almost the vanishing point.

In another case the total albumin was 0.4678 gm. per 100 c.c., and the globulin 0.16 gm. per 100 c.c. (34 per cent.). In still another case there were 18 gms. of albumin per litre, a multitude of casts and renal epithelium, yet at autopsy no evidence of severe trouble.

UNILATERAL NEPHRITIS.—In our series with autopsy no cases of this description occurred. Of 90 cases, in at least 30, or 6 per cent., there was considerable inequality in size of the two organs, yet in all but three cases, or 0.6 per cent., these were large kidneys. In these three cases the combined weights were 155, 190, and 205 gms., and the difference in weight between the two, respectively 45, 50, and 65 gms. In a very interesting case at operation was found unilateral suppurative nephritis.

RENAL ATROPHY.—This may be due to insufficient blood-supply, to cachexia, the anæmias, and especially to advancing age, the “senile atrophy.” It is never great in amount. There are seen microscopically

sclerosed glomeruli, but no great increase in connective tissue. The urine is practically normal, and without albumin.

**Congenital Cystic Kidney.**—The urine in this very rare condition may be normal, or show the picture of the chronic interstitial nephritis with small contracted kidney. The amount of urine is increased, the specific gravity low, with or without a trace of albumin, often much blood. The contents of these cysts are rather interesting, being not at all uniform, and in the same kidney different cysts may have different contents; sometimes clear, watery, almost colorless, or milky or colloidal; sometimes containing urea, even in large amounts, or uric acid, sometimes none. Often cholesterin crystals; colloid or proteid-like masses, rosette masses which resemble leucin have been described.

**Suppurative Nephritis.**—In such cases we have the ordinary symptoms of acute nephritis with albumin of varying amounts, but only a few casts. In the sediment, however, in one case there were a great many red blood-cells and leucocytes. In the other cases there seem to have been very few leucocytes. When many, the urine will be alkaline. Very rarely fragments of renal tissue have been found.

There was recently in the gynecological department a remarkable case of unilateral suppurative nephritis.

In cases of purulent nephritis the amount of pus which is found may be disappointingly small since the kidney with the abscess, either as a whole or in the affected part, may excrete no urine. In the metastatic renal abscesses there are no urinary symptoms as a rule.

**Cancer of the Kidney.**—Hæmaturia is often an early, even the first symptom. It occurs in over one-half of the cases, and is the first symptom in one-fourth. The amount may vary from a very slight trace to a fatal hemorrhage, the hemorrhage may be intermittent or of long duration, the blood fresh or decomposed, and clots even of large size may be voided. Otherwise the urine is practically normal.

**Tuberculosis of the Kidney.**—In a general miliary tuberculosis there are no urinary symptoms as a rule, and when present they are not due to the tuberculosis alone. In tuberculosis of the pyramids, in which case it is common to have large caseous masses which break down and leave a cavity, the so-called "renal phthisis," the urine is similar to that in pyelonephritis. If it be the pelvis which is involved, caseous matter may be found in the urine. If the pelvis be normal there may be no urinary changes. The very early polyuria with or without albuminuria is an interesting feature. Hæmaturia may be the first symptom, and was present in eight of the seventeen cases from this clinic which were reported by Dr. Walker.<sup>163</sup> This early hæmaturia is very seldom a marked or serious feature, and may last for months. It is present both day and night, and bears no relation to the position

<sup>163</sup> Johns Hopkins Hosp. Rep., vol. xii.



of the patient, hence differs from that due to calculus. On the other hand it may be so severe as to be a serious feature. Pus was present in fifteen of the seventeen cases, in little or large amount according to the position of the cavity. Blood-clots are common; tissue detritus and masses about the size of a grain of sand occur, and in them are found tubercle bacilli and elastic tissue. These were present in nine of the seventeen cases. Albumin was present in sixteen, and casts in six of this series. One should not be misled by the perfectly normal urine which may be excreted during the days that no urine comes from the diseased side.

In general, it may be said that in all cases of hæmaturia and pyuria, especially if the urine is acid, tuberculosis of the kidney should be thought of. For diagnosis the tubercle bacilli must themselves be found. But since it would appear that bacilli can be excreted through a normal kidney tuberculosis of other organs must also be excluded. If a focus of disease does not ulcerate into the pelvis of the kidney the entire organ may be destroyed and yet the condition be unsuspected.

**In infarction of the kidney** there is usually a preceding nephritis. In the sediment red blood-cells are usually present, but marked hæmaturia is rare.

In cases of bilateral infarcts, there may be oliguria, and even anuria. An intense albuminuria with sudden onset and rapid disappearance and no abnormal sediment is a very suggestive feature.

**Pyelitis and Pyelonephritis.**—Inflammation of the pelvis of the kidney may be due (1) to an infection ascending along the ureter, or a descending renal infection, or an infection extending by contiguity from neighboring organs; (2) to local causes, stone, cancer, tuberculosis, parasites (*echinococcus*, *amoebæ*, etc.), trauma, floating kidney; or (3) to systemic causes, specific toxins of acute fevers, medicines, etc. It is usually unilateral.

The symptoms are usually masked by those of the general or causative disease, and even when attention is directed to the possibility of a pyelitis there may be no localizing symptoms to indicate it.

The urinary features will depend on the cause. Sometimes there is anuria (due to the reflex influence over the sound kidney). In chronic cases the amount of urine is sometimes even trebled. It is cloudy from the pus, blood, and mucus, and faintly acid unless there is ammoniacal decomposition. It contains little albumin.

In the pyelitis of infancy due to *Bacillus coli* there is often no pus in the urine until a few days after the temperature begins to rise.\*

Microscopically, the urine contains red blood-cells, mucus, pus, various epithelial cells, uric acid crystals, calcium oxalate crystals, fibrin coagula, tissue constituents, and other elements suggesting the

\*Thomson, Quart. Jour. Med., 1910, vol. iii, No. 11.



cause of the trouble, as tumor fragments or parasites. It has been a much disputed question whether from the nature of the epithelial cells the situation of the trouble could be determined. It seems to be generally conceded that the epithelium from the pelvis of the kidney to the urethra is quite uniform. Sahli suggests from observation of one case that in pyelitis it is the cylindrical cells with tails (Fig. 46, a, b) which are especially increased. We have found many of these cells in several cases, but in one very acute case with autopsy the urine contained none. The epithelial cells are often in clusters with strata, presenting the well-known tile arrangement of the tailed club-shaped cells. There will be no casts or renal epithelial cells in case there is no nephritis. In the diagnosis the urine examination is particularly important. The reaction of the urine is usually acid.

Of importance is the variability of the urine, the obstruction of the diseased side causing periods with normal urine, then the appearance of all the elements of the pyelitis.

Various crystals are present, and in the diphtheritic form threads of fibrin and casts of the pelvis of the kidney, or tissue fragments.

In the diagnosis of pyelitis, of greatest importance is the absence of disturbance of micturition, the homogeneous mixture of pus and urine, and the club-shaped tailed cells in groups with a tile-like arrangement.

In **hydronephrosis**, **pyonephrosis**, and **uronephrosis**, the urinary changes (apart from pus) are in amount of urine, the periods of oliguria alternating with polyuria, and its constituents, depending on the health of the cortex.

**Renal Calculus.**—During the renal colic the urine may be normal or anuria total, but when the obstruction is relieved, blood, mucus, and pus appear.

Independent of colic, hæmaturia is a common symptom (especially of oxalate stones), sometimes with the passage of a clot of blood; sometimes the hemorrhage is profuse, especially early. Later the symptoms are those of pyelitis.

With *ureteral calculi* occur hæmaturia and oliguria, followed by polyuria. The oliguria is a feature in about 25 per cent. of all cases, and even anuria in 16 per cent.<sup>164</sup>

**PARASITIC DISEASES OF THE KIDNEY.**—In *echinococcus disease* the only urinary symptoms are in some cases a mucous catarrh of the pelvis of the kidney, which later may be purulent pyelitis or gangrene. If the large cyst ruptures through the urinary tract, there is the sudden appearance of a watery fluid (or soapy or milky or bloody), and while the cyst is discharging the hooklets, scolices, fragments of membrane, etc., may be found in the sediment.

<sup>164</sup> See Schenck, Johns Hopkins Hosp. Rep., vol. x. p. 477.

(For other parasites see page 311.) In the *Bilharzia infections* (see page 311) pyelitis and even renal atrophy may result.

#### FUNCTIONAL RENAL DIAGNOSIS

During the past few years the amount of work in this very promising field has been enormous (over fifty theses and articles have appeared in about seven years), but, sad to say, is rather unfruitful.

The discrepancy between the anatomical condition as found at autopsy and that which would be supposed from the urinary examinations is proverbial. Small contracted kidneys of less than one-half or one-third normal size may excrete a urine normal in amount and specific gravity, with but a trace of albumin and a few casts; when the urine was full of casts and a great amount of albumin, no clear evidence of nephritis may be found; again, persons have died in uræmia, than which there is no better evidence of renal insufficiency, and yet the urine contained but a trace of albumin, and at autopsy the renal changes were very slight. Evidently the time-honored chemical and microscopical methods are too gross.

The next problem was to find a more delicate test than these to determine a renal condition which would be unsuspected if ordinary tests were used, and which would also allow of diagnosis before the anatomical changes were evident; also a test which would prophesy an oncoming uræmia. Two of the most delicate tests of physical chemistry were chosen,—cryoscopy and electrical conductivity of the urine and blood. It was hoped that the results of this work would be more in accord with pathological findings on the one side, and when these would be deceptive could we see the kidney, would show a definite renal insufficiency did it exist clinically. In connection with these tests is often used the sodium chloride test.

The third line of work disregarded the anatomical condition of the kidney altogether, and asked as to its functional ability. For, a well-compensated severe lesion is manifestly of less immediate danger than a poorly or non-compensated slight lesion. In these tests an extra demand is suddenly made on the kidneys and its response determined. Such tests are the sodium chloride, the methylene blue, the rosanilin, the phlorizin, salicylic acid, and potassium iodide, *et al.*, tests.

Before describing these tests it is well to emphasize the fact that the toxins so evident from their results in nephritis are as yet unknown; and that nephritis is more a general disease, the kidney features playing only one part. All that can be done is to test the way in which the kidneys behave toward known substances, or perform their ordinary duties, or the unusual which we impose upon them, and from this by analogy surmise how well they perform their other

functions. Another point to be remembered is that the function of the kidney is not as well understood by the physiologist as the clinician seems to assume when he uses the methylene blue test to test the "epithelial filter," the salicylic acid to test the "glomerular filter," and phlorizin to test the "glandular activity" of the renal epithelium.

Most admit that one test is never enough; that all must be used to get a good picture, and even with all one is dissatisfied (see the many French theses of 1902 to 1903, among them Miorgec, Lyon, 1902, and Jouffray, Lyon, 1903).

We wish to warn workers that nearly all these functional tests make an unusual demand on the kidneys, to which they may not be able to respond, and disastrous results follow.

**Cryoscopy, Freezing Point of the Urine.**—By means of this determination one hopes to find out how many molecules the kidneys are excreting on the one hand, how well they keep the serum free from an accumulation of these molecules on the other. Of these two values the latter is the more important, since the former will depend on the diet, etc. The latter is an index of the success the kidneys have in keeping the plasma free from the products of catabolism. It is not what the kidneys eliminate, but what they should but do not, which it is of interest to determine, and the examination of the blood gives some clue as to this.

The determination of the freezing point of solutions is a well recognized method of physical chemistry to determine the molecular weight of the substance in solution; also the degree of disassociation of the molecules. The method, therefore, is, from the chemist's point of view, one of great importance. But even when the problems are of the simplest nature, when dilute solutions of a single and pure salt are used, and with but the simplest point to determine, the method requires experience, skill, and the due regard to a good many factors which can modify the results. It is hard to see, therefore, how this method can be applied with much success to complex fluids like the urine or blood, in which is dissolved a great variety of bodies of widely different nature, some unknown. The belief was, however, that from very slight differences in the freezing point of these fluids important deductions could be made concerning the functional ability of the kidney. It must, of course, be remembered that the changes in the freezing point are exceedingly slight, varying in many cases but a few hundredths of one degree; a variation which the physical chemist considers slight is that from which the clinicians draw conclusions. The clinician, therefore, should use even greater care than the physicist in using this method, and at least as delicate instruments. The reverse has, however, been true. Cheap "clinical" instruments which

cannot be accurate are placed upon the market, and a multitude of freezing point determinations made with a disregard of the chances of error which must exclude the possibility of correct results even in a much simpler problem.

The principle of cryoscopy is this: When a substance is dissolved in a liquid, the freezing point of the latter is lowered to a degree which in a general way is proportional to the concentration of the solution. The lowering of the freezing point of a given liquid, for instance of water, by a 1 per cent. solution of a substance, is called the "specific depression" of the freezing point of that liquid by that particular substance, and a 2 per cent. aqueous solution of that same substance should cause double that depression. It has been found that the depression of the freezing point bears no relation to the molecular weights of the substance, but to the number of molecules in solution. In other words, equal numbers of molecules when dissolved in equal quantities of a given liquid will produce the same lowering of the freezing point whether the molecules are of the same or different nature. By "molecular depression" is meant that caused by a solution in 100 gms. of a given liquid of a number of grammes of the substance in question equal to its molecular weight.

The above statements have one very important exception. They hold for those bodies which are not disassociated in solution. In the case of electrolytes—inorganic acids, bases, and salts—in dilute solution the depression of the freezing point is at least twice that of equivalent amounts of organic substances, since electrolytes are disassociated, and each ion has the same effect on the freezing point as a complete molecule. The difficulties are at once evident. In the blood and in the urine we will have a large number of bodies in solution in proportions varying considerably, probably in conditions of association which we can neither control nor determine. The result obtained by the clinical chemist is therefore a depression of the freezing point produced by a resultant in this mixed solution of really unknown nature.

Of course, this result could be of greatest value if it were empirically shown to be so, even though the phenomenon could not be well explained.

The question is, then, Does experience prove cryoscopy a valuable clinical means of determining renal sufficiency, or is the clinician only playing with a scientific toy which has for him only the appearance of truth?

**METHOD.**—The apparatus (see Fig. 62) consists of an exceedingly delicate thermometer, *a*, and the best is none too good, which reaches almost to the bottom of a test-tube, *b*, scrupulously clean, containing the solution whose freezing point is to be determined. This

test-tube is enclosed in an air-jacket, *c*, which is surrounded by a freezing mixture of ice and salt.

The Beckmann thermometer is generally used. The makers of this instrument now supply one for freezing points alone, which is a distinct advantage over the Beckmann for both freezing and boiling points. In the freezing mixture is inserted a smaller thermometer, *d*, that its temperature may be controlled, and a stirrer, *e*, that it may be kept well mixed. Before and after every determination the zero of the thermometer must be determined for distilled water. Of the same water the second zero will often be slightly lower than the first since the freezing has purified the water of carbon dioxide. The lower of these values is the zero of the experiment.

The fluid whose freezing point is to be determined must cover the whole or at least over two-thirds of the bulb of the thermometer. In any accurate apparatus this will require at least 10 cc. of fluid, and those instruments advertised for 5 cc. are to be regarded with great suspicion. The temperature of the freezing mixture should not be more than  $1^{\circ}$  to  $3^{\circ}$  lower than the freezing point of the fluid to be determined, since undercooling may give too low a point.

The method used by the physical chemist is as follows: Distilled water is put into the tube, *b*. The water is allowed to cool somewhat below the freezing point and then by means of the stirrer, *f*, stirred vigorously until ice begins to form. The thermometer will then rise a little and remain con-

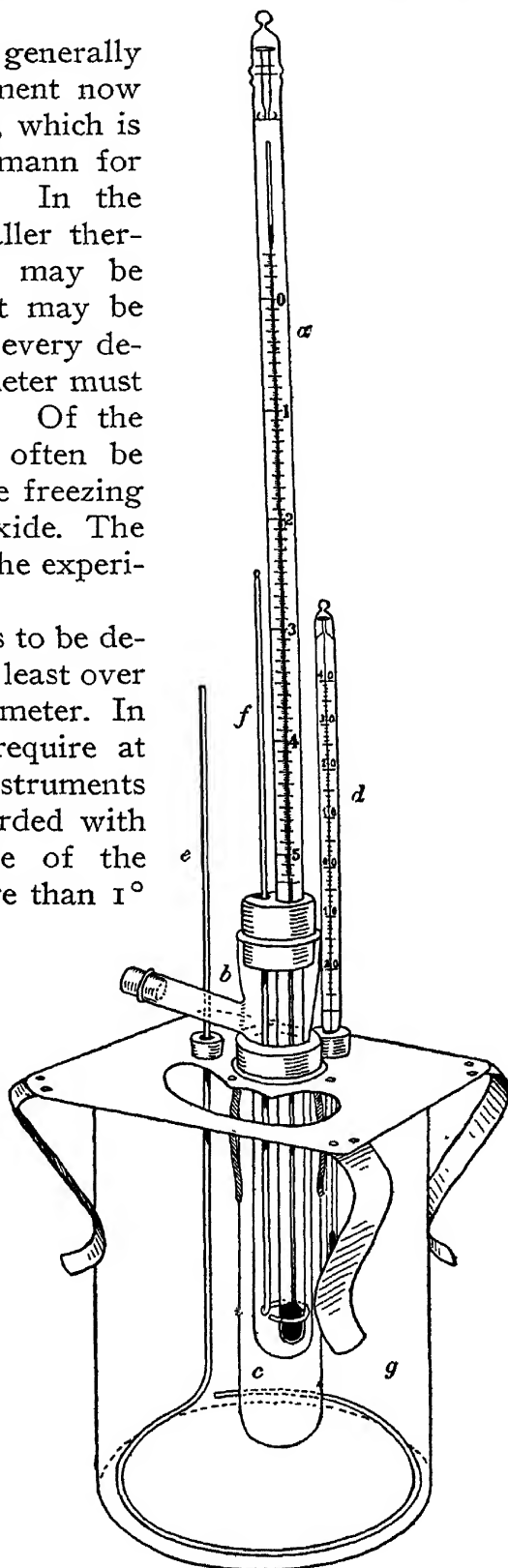


FIG. 62.—Apparatus for freezing-point determinations. *a*, thermometer graduated to  $0.01^{\circ}$  C.; *b*, tube for fluid to be investigated; *c*, air-chamber; *d*, thermometer for freezing mixture; *e*, stirrer for freezing mixture; *f*, stirrer for fluid; *g*, jar for freezing mixture.

stant. The highest temperature is the true freezing point of the water. This is to be repeated several times and the lowest used. The test-tube containing the water is then removed and that containing the fluid in question substituted. This also is stirred until ice begins to form, at which time the thermometer will rise, remain stationary for a few moments, and then fall. The highest temperature is the one to be recorded. The rise is caused by the liberation of the latent heat of crystallization, and the subsequent fall due to the concentration of the solution, since the ice formation removes just so much solvent. That this highest point may be the correct one it is necessary that the freezing mixture should not be too cool, or we may get "under cooling." If ice does not form, a very small crystal of ice should be inserted into the tube, which will start the freezing at once.

Certain points must be observed with care: The repeated correction of the zero point for each determination already mentioned. The bulb of the thermometer would better not rest on the bottom of the test-tube, or there may be too much ice formed here; this error is, however, small. The stirrer should theoretically be moved by machinery, since its motion should be perfectly rhythmical and timed by a metronome; irregular stirring results in error. Since the cooling should be slow and the stirring take fifteen to thirty minutes, it is impossible to do this by hand.

But this careful performance of the test is seldom carried out. As a rule, the clinician considers the determination easy and remarkably rapid, and for him it is, but we are glad to say that long series of observations have been made by those who have observed all the above precautions, and their work (mentioned later) is alone of value in judging the method.

Some go so far to the other extreme as to say that the molecular concentration may be easily determined by multiplying the last two figures of specific gravity by 0.075.

To determine the freezing point of *urine* it is better to use a twenty-four hours' mixed specimen than to test separate voidings. The vessels in which this is kept should be perfectly clean. If rich in urates the freezing point of the cloudy fluid may be determined, and to the result  $0.04^{\circ}$  added or the fluid may be cleared by centrifugalization and the freezing point of the clear fluid and sediment determined separately.

Linossier and Lemoine<sup>165</sup> have shown that the position of the patient is important, since excretion is very different when erect from when in bed, being even five times as much in the latter position.

*Blood*.—This is best obtained through a canula in the median vein at the bend of the elbow. One must be sure, however, that the cir-

<sup>165</sup> Compt.-rend. Soc. Biol., vol. lv. pp. 469, 605.

culation is perfectly free before the blood begins to flow, since the venous stasis alters the freezing point considerably. Serum, defibrinated blood, or the pure blood may be used with theoretically the same results. Most of the better workers, however, prefer the serum. The defibrinated blood has the error of the high  $\text{CO}_2$ -content which can make a difference of  $0.02^\circ$ . It is better to let the blood clot in an air-tight vessel, or to condense the clot by centrifugalization. At least 40 cc. of blood in the first case are necessary in order to get enough serum; in the second case, from 25 to 30 cc. Two determinations should be made, the observer being sure that the serum is perfectly thawed between them.

In the following paragraphs  $\delta$  = the depression of the freezing point of blood;  $\Delta$  that of urine.

The results obtained were at first considered promising, but more recently the method has not received the same approval. Koranyi, who brought the method into prominence in 1896, was exceedingly sanguine. He considered that  $\delta$  was very little lowered, that is, below  $0.56^\circ \text{C.}$ , in anæmias and fevers which do not affect respiration, but was lowered considerably by any disease causing insufficiency of respiration, or renal insufficiency, or both. He thought that cryoscopy would help the diagnosis between typhoid fever and pneumonia, since in the latter  $\delta$  is lowered. In renal disease, for  $\Delta$  to be high (*i.e.*, abnormally near  $0^\circ$ ), means renal insufficiency. From cryoscopy of the urine he believed in anæmias  $\Delta$  to be less than  $1.4^\circ$ , a point which is considered normal. In nephritis it is very little less, but in anæmias, in renal insufficiency, and in malnutrition is molecular oliguria present (about  $-0.80^\circ \text{C.}$ ). It is seen that he expected much from this method.

The freezing point of normal serum in man is much less variable than that of any other animal, it being from  $-0.55^\circ$  to  $-0.57^\circ \text{C.}$  In case both kidneys of an animal be removed, the effect on the blood is enormous, even  $-0.75^\circ \text{C.}$ , but a partial injury has a less effect, Koranyi stating that one-half the renal parenchyma could be destroyed before any effect was shown.

The remarkable ability of the body to keep  $\delta$  constant is seen in the case mentioned by Kümmel, in which  $\delta$  was  $-0.57^\circ \text{C.}$  before an intravenous injection of 2000 cc., and four hours later exactly the same. This writer in a long series of observations found almost always when there was unilateral disease a lowering of  $\delta$ .

The amount of work done with cryoscopy is enormous, at first quite promising, but later less so. Our own experience is not sufficient to report. We think the present status well given by Schoenborn (Wiesbaden, 1904), who has done some very careful work with this method. Forty-two cases of nephritis were studied and

the various formulæ applied. His conclusions are that the ordinary methods of urine examination, the microscopical and chemical, are of more value than cryoscopy of the urine and blood; that clinically the latter can be omitted but the former never. He found that from cryoscopy no information was gained concerning the nature of the processes, the functional ability of the kidney, or the severity of the disease. It is true that the majority of cases will conform to certain rules, but one is so often deceived, the number of exceptions to these general rules so great, and in all probability their number will increase so greatly on further work, that the value of this alone is to be much doubted. As a delicate method of predicting any future change of the patients' condition or of detecting a latent nephritis which cannot be recognized by the ordinary clinical methods, he doubts that it is of any value.

In most of his cases  $\Delta$  fell within those limits usually considered normal, and some of these were the severe ones. In the severest cases  $\delta$  did drop, yet gave no indication of the nature of the lesion.

In uræmia it is granted there is the most serious renal insufficiency, and hence this is the condition by which the method can be best tested. He concluded from the study of 88 cases, including nine of his own, that cryoscopy in some cases shows a normal condition, in the majority of cases the same condition as of nephritis without uræmia, and in only a few cases a striking abnormality, as, for instance,  $\delta = -0.975^{\circ}$  C. Engelmann on the contrary reported a series of 36 cases,  $\delta$  averaging  $-0.664^{\circ}$  C. Again, in cases with no suspicion of renal insufficiency  $\delta$  could be very high, even  $-0.67^{\circ}$  C.

In surgical cases with a gross bilateral renal lesion destroying all the functions a good parallelism between the lowering of  $\delta$  and the development of the uræmic symptoms may be followed (Kümmel), but in medical cases this is not so true, since the lesion, whatever it may be, in some way brings about uræmia while those renal functions which we can measure are still well performed.

According to the medical men, therefore, the cryoscopy of the blood and urine gives some idea of the osmotic activity of the kidneys which is not given by any other method;\* this idea enlarges in some cases the clinical picture and is of some value, but it rarely gives any information which could not be learned from the ordinary methods. It never foretells an oncoming uræmia, or decides the nature, the prognosis, or the results of therapy, or reveals a condition not already suspected. It is a method which requires a great deal of practice, and the accurate control of a great many conditions, both in its performance and in the previous care of the patients, such as diet, etc. The results obtained by the skilful are interesting, in some cases valuable, in the majority disappointing, and in some deceptive. The cryoscopy of the



blood alone demands considerable experience, and gives little more than would be expected from the clinical observation of the case, and often not that much. The cryoscopy of the urine is of very little value even when greatest care is taken concerning diets, fluid, etc. Schoenborn examined 52 cases of non-nephritics with many diseases, and found it of practically no value whatever in differential diagnosis or to follow the results of therapy. He admits that the conditions of the kidney and cortical vessel are the most important factors governing  $\delta$  and  $\Delta$ , but there must be other unknown factors in some cases still more potent. From the study of all these cases no general deduction could be made.

Strauss,<sup>166</sup> who carefully governed the food and water intake, also had found cryoscopy of the urine unsatisfactory, and evidently more cases atypical than typical. For the value of cryoscopy in hepatic diseases, see Ferrannini.<sup>167</sup> Cryoscopy has also been used for the quantitative determination of albumin and sugar, but without much success.

Among the various FORMULÆ used in the hope of getting constants for use are the following:

$\Delta$  = depression of freezing-point of urine ;  $\delta$ , that of blood.

$\frac{\Delta \times \text{amt. of urine (V)}}{\text{body weight (P)}}$  (Claude and Balthazard) "the total molecular diuresis," the index of "glomerular filtration;" this was found inconstant.

$\Delta \times \text{amt. of urine}$ , Strauss' "valence value," is useful; the "molecular diuresis" of Koranyi.

$\frac{\Delta \times \text{amt. of urine}}{61.3}$  "NaCl equivalent" of Koranyi ( $\Delta$  of 1 per cent. NaCl = 0.613).

$\frac{\Delta}{N \text{ per cent.}}$  (Waldvogel).

$\frac{\Delta}{\text{Sp. gr. of urine.}}$

$\frac{\Delta}{\text{NaCl}}$  (Koranyi).

$\frac{\Delta}{\delta}$  is of value indicating the permeability of the epithelium of the tubules.

$\frac{\Delta \times \text{amt. of urine}}{\delta}$  (Bernard).

$\frac{\delta V}{P}$  an approximate index of urinary toxicity.

**Electrical Conductivity.**—This method of physical chemistry also has been appropriated by the clinician in his desire to learn something

<sup>166</sup> Zeitschr. f. klin. Med., 1902, vol. xlvii. p. 39.

<sup>167</sup> Centralbl. f. inn. Med., vol. xxiv. p. 273.

about the functional ability of the kidneys. By electrical conductivity is meant the reciprocal of the resistance which a certain amount of a solution between two platinum electrodes of given size and given distance apart offers to the passage of a current of known strength. This is really a measure of the number of electrolytes in solution, that is, of the disassociated ions. It is not affected by such bodies as albumin, sugar, urea, which are not disassociated, and hence is practically a measure of a few salts in the blood and urine, especially the chlorides. This also is a method which is very valuable to the physical chemist of experience to determine simple points concerning simple solutions, and even for him to draw deductions requires a full understanding of all the conditions present in the determination. It would seem, therefore, like one working in darkness to apply this very delicate method to solutions, such as the blood and urine, which contain an unknown mixture of various bodies which, because of this mixture, may not disassociate as they would were they in pure dilute solution. Yet if found by experience to be of value, no theoretical objection should be urged against it.

**METHOD.**—The method of Kohlrausch is that usually used. By this method an alternating current is passed between platinum electrodes through the solution whose conductivity it is desired to study. The resistance is balanced on a Wheatstone bridge against a rheostat, and the point of equilibrium determined by means of a telephone. The urine, for instance, is placed in a U-shaped tube of known length and holding 4 to 8 cc. of fluid, in which are immersed the platinum electrodes, which must be very carefully prepared and covered with platinum black in order to secure a sharper minimum in the telephone. This vessel containing the urine is placed in a thermostat, the temperature of which does not vary over  $0.1^{\circ}\text{C}$ .

For detailed description of the method standard works on physical chemistry should be consulted. But this method has been popularized, and on the market are instruments for "clinical use" which allow of a rapid determination and, which is their chief advantage, require a very small amount of urine (1 to 2 cc.).

The conclusions thus far are that the electrical conductivity in the case of the blood and the urine is somewhat parallel to the freezing point and gives nothing definite. Many abnormalities are found, but one does not know how to interpret them. For this reason we refrain from a more elaborate description of the method.

**The delayed excretion of urea** is an old criterion of functional renal ability. In acute disease it may take three to six days to excrete the urea formed from one day's meals; in chronic nephritis and renal tuberculosis, two days. During this delay the urea accumulates in the blood, and may be determined quantitatively (see page

575). When it is increased tenfold (*i. e.*, to 0.3 per cent.), there is danger of uræmia (Herter).

**Chloride Excretion.**—The rapidity with which the kidneys can excrete a considerable amount of sodium chloride is taken as a test of its functional ability, or, as some specify more particularly, of the “glomerular sufficiency.”

This test of “alimentary chloruria,” recommended by Claude and Manté,<sup>168</sup> is used usually in conjunction with cryoscopy. It consists in placing the patient on a milk diet (3 l. per day), and after the third day adding sodium chloride, 10 gms. a day, dissolved in 125 cc. of water, which are given in three portions on each of four consecutive days. The daily output of the chlorides is determined.

Normally the excretion begins at once and ends abruptly when ingestion ceases. The water output is increased, but to a less degree. The other constituents of the urine are also slightly increased and continue so for a longer time than the increase in Cl.

These writers divide renal cases into four groups: the first, of those who react exactly as normal persons do; these bear their lesion well. In the second group the increased chloride excretion causes a considerable increase of other urinary constituents, especially the nitrogenous bodies, which continues several days, as if the sodium chloride had acted favorably on the kidneys. In the third group the increase in chlorides is delayed, reaches a slow maximum, and continues two to four days after ingestion ceases. There is also an increased output of other bodies, but it is less in this than in the second group. In the last group the ingestion of salt causes no increased Cl output, no (or a slight) diuresis results, and the excretion of other bodies is increased. The prognosis of the third group is bad; of the fourth, fatal.

Yet further work has shown that there is nothing constant in this excretion in renal disease; there are too many exceptions, and not always can these be interpreted.

**THE DILUTION TEST OF ALBARRAN.**—The excretion of water is so important a function of the kidney that it is natural to think that variations in this would be a more satisfactory test of renal permeability than variations in the excretion of the many substances mentioned above which are quite foreign to usual diet. Briefly stated, this test is as follows: The  $\Delta$  of the patient's urine is determined, and then he is asked to drink a large amount of water (from 1 to 2 litres). The time of the appearance, and the duration of the resulting polyuria or of the  $\Delta$  are noted. If one seeks to determine the combined efficiency of the kidneys, the patient is asked to void every half hour, and the urine voided each time is examined separately. If one seeks to determine the relative efficiency of the kidneys, the ureteral catheters are left in place for at least three hours, and the urine from each is collected in half-hourly portions.

The dilution of the urine may begin during the second half hour, reaches its maximum in two or three hours, and lasts five or six

<sup>168</sup> Arch. gén. de méd., 1902, vol. viii, n. s., p. 129.

hours. In parenchymatous nephritis the ability to free the body from the excess of water seems less than in cases of contracted kidney. When the kidneys are not equally diseased the excretion of the diseased kidney is more uniform than that of the more normal organ; so that in case we are separating the urines of the two kidneys the more normal side will show the dilution first and more markedly. This test has never proved itself of great value. Even in the normal person we find no uniformity in the time of appearance, or in the duration of a polyuria following the ingestion of large amounts of water. Indeed, a normal person, and much more an invalid, may show no polyuria which is demonstrable under the conditions of the test. Again, there may already be a polyuria of the diseased side which will mask any increase of the output of the more normal side. And, lastly, three hours are a long time to allow the ureteral catheters to remain in place.

Among the other terms used are "glomerular insufficiency," which means the retention of water and NaCl, the excretion of a scanty concentrated urine, the inability to excrete a dilute urine. "Tubular insufficiency," the retention of nitrogen and phosphates, the excretion of a normal amount of dilute urine, the inability to excrete a concentrated urine.

**Renal Permeability.**—THE METHYLENE BLUE TEST (of Achard and Castaigne) is supposed to test the "epithelial filtration." This dye may be given by mouth, 0.1 gm. in a capsule, or 0.05 gm. subcutaneously, that is, intramuscularly (1 cc. of a 1:20 solution of methylene blue). This latter is the better method, since the disturbing factors on the part of the digestive canal are eliminated.

The dye is eliminated, first as a colorless chromogen in fifteen to thirty minutes after a subcutaneous injection, and as a greenish-blue pigment three to five minutes later. To appear first only after an hour is pathological. Normally the excretion reaches a maximum in from three to four hours and lasts from thirty-five to fifty hours (forty-eight to sixty); the chromogen is last to disappear. About one-half is eliminated in the first twenty-four hours.

As soon as given the bladder is emptied and the urine examined at stated periods, first in half an hour, then hourly. It is boiled with acetic acid to oxidize the chromogen. The time is measured from the appearance of the colorless pigment thus made evident until this chromogen disappears.

It has been noted that toward evening the elimination ceases, to be resumed the following morning. Others show a "polycyclic" elimination, *i.e.*, a very irregular curve with periods in which none is eliminated. This is seen in interstitial nephritis and various neurotic

conditions, but is said to be especially true of "hepatic insufficiency" (Pugnat and Revilliod).

But the delay or non-delay of elimination is not of great importance, since even a small amount of normal renal tissue in an extensively diseased kidney will excrete some at the normal time, hence the amount excreted is considered of greater value. The results of these tests of the renal permeability in the hands of its friends, especially the French, may be stated as follows: The delayed and protracted excretion depends directly on the acuteness of the process; in chronic parenchymatous nephritis, as a rule, the permeability is good; also in some cases of chronic interstitial nephritis. Others say that in nephritis the excretion is always delayed; that when elimination begins in normal time either the kidneys are sound or the lesion local.

In some cases the excretion is delayed and lasts longer than normal, even seven days, and the total output is less. This is seen in renal atrophy, and is considered a sign of "diminution of the excretory surface" (Achard). In some cases "with epithelial lesion predominating" the kidneys seem abnormally pervious, the excretion begins in a very few minutes, and continues but about twenty-four hours (Bard). In a third group it begins late, but lasts only a short time; *e.g.*, Widal's case began at the end of five hours and lasted but two hours. The greatest abnormality is therefore in interstitial nephritis, while in parenchymatous or amyloid the test may show a normal or abnormally rapid output.

Walker found that in obstruction of the lower urinary tract, as that caused by hypertrophy of the prostate gland, there may be delay in the excretion of the methylene blue, and the dye may not appear at all.

Herter, while admitting that a delay means disease, agrees that some patients show periods of normal output. He denies that in any case is there a shortened period of excretion.

From the first the test has been severely criticised (especially by Germans). Too many cases with kidneys found at autopsy to be the seat of extensive disease reacted normally or with abnormal rapidity. In uræmia even the test may show normal permeability (but see Bard's case). The variations are not marked enough; it is not reasonable to judge of the permeability of the kidneys to normal or abnormal constituents from the excretion of this very abnormal body, and since the permeability for methylene blue is known to be different from that for known bodies, it may be very different from that for the toxine causing uræmic symptoms; while, on the whole, in nephritis there is delayed and abnormally protracted excretion, yet the various forms of renal disease show little or no difference; the permeability is altered even in neuroses. In some cases the dye is entirely destroyed

in the body, not even the chromogen appearing in the urine. Under normal conditions only about 50 per cent. of the dye is excreted through the kidneys. And lastly, even the warmest friends of the test have modified it, and now emphasize the total output of the dye as of most importance, which causes one to mistrust the test, for this quantitative determination is uncertain, hard, and it is not at all certain that this amount would be any good index of renal activity.

**INDIGO-CARMINE TEST (VOELCKER AND JOSEPH).**—Twenty cubic centimetres of a 0.4 per cent. solution of indigo-carmin are injected into the gluteal region. Normally, the color of the urine will change to a greenish-blue in from 10 to 15 minutes after the injection, and will return to normal in about 12 hours. That is, the elimination of this dye is more rapid than that of methylene blue. Yet only about 25 per cent. of this dye is excreted through the urine. The rest cannot be accounted for.

**ROSANILINE (LEPINE).**—One cubic centimetre of a 1 per cent. solution of rosaniline is injected into the gluteal region. The excretion of the dye begins in about 30 minutes, reaches its maximum in 2 or 3 hours, and continues from 20 to 24 hours. Since from 65 to 95 per cent. of this dye is excreted in the urine, this test theoretically has more in its favor than have the preceding color tests.

**Salicylic Acid Test.**—This test was adapted to clinical use by Widal and Ravant as a measure of renal permeability. One cc. of a 30 per cent. solution of sodium salicylate is injected (with a little cocaine to reduce the pain) intramuscularly. The urine is examined at the end of half an hour, then hourly, with 10 per cent.  $\text{Fe}_2\text{Cl}_6$  solution. Colorimetrically can the amount excreted be determined.

Normally the violet color is given by the urine voided at the end of half an hour, even of fifteen minutes; it reaches a maximum in from one to three hours, and disappears in from eight to twelve hours. The amount excreted (*i.e.*, the per cent. of the total) in five hours is taken as a sort of standard. The excretion is supposed to be through the glomeruli and governed by physical laws alone. In the various forms of nephritis the excretion may begin within the first half-hour (yet perhaps in the first fifteen minutes), and reach a maximum at the same time for all. In some of the cases of parenchymatous nephritis there is the same duration of excretion and the same relative output in five hours as normal, but in some interstitial cases the output is continued over a long time and is less in amount; yet in even the very few cases reported there are striking exceptions.

It has certain advantages over the similar POTASSIUM IODIDE test, since it is simpler and more rapid.

If a QUANTITATIVE ESTIMATION be desired, Ziegan<sup>169</sup> recommends the following: To from 30 to 50 c.c. of urine in a graduated mixing cylinder are added 1 c.c. of dilute  $\text{H}_2\text{SO}_4$  and 50 to 80 c.c. of ether, and shaken three to five minutes. This is allowed to settle. One-half the ether extract (with the salicyluric acid) is removed by a pipette and poured into a separating funnel. To it is added 2 per cent.  $\text{Fe}_2\text{Cl}_6$ , till the color does not change. It is then poured into a glass suitable for color determinations. Into another glass of exactly the same character are poured an equal volume of ether, the same amount of 2 per cent.  $\text{Fe}_2\text{Cl}_6$  which was added to the first glass, and then 0.1 per cent. of salicylic acid from a burette until the same shade is obtained.

For the quantitative estimation of KI see Singer.<sup>170</sup>

**Phlorizin Test.**—This test of the “secretory ability” of the renal epithelium, rather than of its “permeability,” [in the latter function osmosis is supposed to play the important part, in the former, none] was proposed by Achard to replace the HIPPURIC ACID TEST (the ability of the kidney to transform benzoic to hippuric acid; a theoretically good test of renal functional ability, but clinically useless since the determination of hippuric acid is so inexact). The phlorizin test is based on the generally accepted view that phlorizin diabetes is due to the specific excretory activity of the renal epithelium and that a diminished or absent glycosuria means disease of these cells. One c.c. of a fresh 1:200 solution of phlorizin (hence 0.005 gm.) is injected subcutaneously (a small dose is chosen which will produce glycosuria in only normal kidneys). The bladder is emptied, then sugar tested for at fifteen-minute intervals. In normal persons sugar will appear in from one-half to one hour, and be present in the urine for from two to four hours. The quantity eliminated is from 0.5 to 2.5 gms. of glucose. In nephritis, as a rule the amount of sugar eliminated is below 0.5 gm., while in some cases none is excreted. This test is now considered unreliable. Normal persons sometimes do not react at all, and the variations in the reaction which do occur may vary out of all proportion to those of the renal condition. Abnormalities in the test may not always indicate renal lesion, but more or less functional disturbance of that organ. The test does not permit one to separate the various forms of nephritis, the “hypoglycosuria,” and “analglycosuria” occurring with about equal frequency in all forms. Yet it is a test of renal activity and tests a quite different function from the others.<sup>171</sup>

Rowntree and Geraghty \* have proposed a test for the functional

<sup>169</sup> Centralbl. f. inn. Med., 1903.

<sup>170</sup> Zeits. f. klin. Med., 1903, vol. xlviii, p. 157.

<sup>171</sup> Pognat and Revilliod, Arch. gén. de méd., 1902, vol. viii, p. 19.

\* Jour. of Pharm. and Exp. Therap., July, 1910, vol. i, No. 6.

activity of the kidneys which promises to prove the best yet suggested. The following description of the method is given practically in the authors' words. The reagent used is PHENOLSULPHONEPHTHALEIN, the solution of which is made up as follows:

Six-tenths of one gram of phenolsulphonephthalein and 0.84 c.c. of  $\frac{2}{N}$  NaOH solution are added to 0.75 per cent. NaCl solution. This gives the mono sodium, or acid, salt which is red in color, and which is slightly irritating locally when injected. It is necessary, therefore, to add two or three drops more of the  $\frac{2}{N}$  hydroxide, a quantity sufficient to change the color to a beautiful Bordeaux red. This preparation is non-irritating.

Twenty minutes to half an hour before administering the test the patient is given 300 to 400 c.c. of water, in order to insure a free urinary secretion, otherwise delayed appearance may be due to lack of secretion.

Under aseptic precautions a catheter is introduced into the bladder, and the bladder completely emptied. The time being noted, 1 c.c. of the above described solution containing 6 mg. to the 1 c.c. is administered subcutaneously in the upper arm by means of an accurately graduated syringe. The urine is allowed to drain into a test-tube in which has been placed a drop of 25 per cent. NaOH solution, and the time of the appearance of the first faint pinkish tinge is noted. From patients without urinary obstruction the catheter is withdrawn on the appearance of the drug in the urine, and the patient is instructed to void into a receptacle at the end of one hour, and into a second receptacle at the end of the second hour. A rough estimate of the time of appearance can be made by having the patient void urine without the use of the catheter at frequent intervals. In prostate cases it is wise to have the catheter in place until the end of the observation. The catheter is corked on the appearance of the drug in the urine, and the cork is removed at the end of the first hour and at the end of the second hour, the bladder each time being thoroughly drained. On many of the patients of this type on whom the observations have been made a retention catheter has been used as a part of the routine treatment on account of the residual urines.

Each sample of urine is measured, and the specific gravity taken. Sufficient NaOH (25 per cent.) is added to make the urine decidedly alkaline, in order to elicit the maximum color. The color displayed in the acid urine is yellow or orange, which immediately gives place to a brilliant purple-red when the solution becomes alkaline. This solution is now placed in a litre measuring flask, and enough distilled water added to make the volume exactly 1 litre. The solution is then thoroughly mixed, and a small filtered portion taken to com-



pare in the Duboscq colorimetre with the standard which is used for all these estimations. The Heliger hæmoglobinometer has been found almost as satisfactory as the colorimetre for this determination.

The standard solution, used for comparison, consists of 3 mg. of phenolsulphonephthalein (or  $\frac{1}{2}$  c.c. of the solution used for injection) diluted up to 1 litre and made alkaline by the addition of only one or two drops of 25 per cent. NaOH solution. This solution is a beautiful purplish-red and retains its intensity of color for weeks or for an indefinite period. One solution, therefore, serves for an immense number of tests.

One cup of the colorimetre (right) is half filled with this standard solution which has just been described, and the plunger lowered so that the indicator reads at 10. A variable quantity (depending on the intensity of the color) of the diluted urine is placed in the other cup, and the plunger manipulated until the two halves of the field are of an identical intensity of color. The indicator of the left plunger is now read, the fraction, as indicated by the Vernier scale, being taken into account. The estimation of the quantity of dye present is then a question of simple arithmetic. For instance, the left side reads at 20, the standard being placed at 10. In other words, it takes a column of fluid twice as long to give the same intensity of color as that of the standard, which, of course, shows that the solution contains only half as much dye. To obtain the percentage of dye excreted in the urine, compared with the amount in the standard solution used for comparison, it is necessary to multiply the reading of the standard by 100 and divide by the reading indicated for the solution containing the urine. To return to our example, we have  $\frac{10 \times 100}{20} = 50$  which indicates that there is 50 per cent. as much drug in the urine as in the standard solution used for comparison.

The standard for comparison described above has been chosen arbitrarily because of the beautiful pink color which is obtained when the indicator stands at 10. At first doses varying from 3 to 60 mg. were injected, but it was found that the larger doses gave a color so intense that great dilution became necessary for quantitative colorimetre estimation. Doses of 6 mg. were selected as most satisfactory for the majority of cases. We have compared the amount of drug in the diluted urine with that of the standard for comparison, but, if we wish to estimate the amount of drug excreted as compared with the amount of drug administered, we must compare the amount excreted with 6 mg. rather than 3 mg., which is present in the solution for comparison. In the example given above we should have 50 per cent. of the 3 mg. or 25 per cent. of the 6 mg., which was the amount injected; so that the excretion is 25 per cent. of the amount ad-

ministered. It is possible to detect a difference of 0.04 mg. of phenol-sulphonephthalein by this method.

An objection raised against the quantitative estimation of the dye substances by colorimetric methods is the influence exerted by the normal coloring matter in the urine. In this test this difficulty does not exist at all, or where it does exist it is easily overcome. The brilliant color of this dye is not influenced perceptibly by a small amount of urinary pigments. Experiments have shown that amounts of urine varying up to 200 cc., and in some cases 250 cc., can be added to the dye, and this diluted with distilled water up to 1 litre, without interfering with the reading. During the test a polyuria is present if the patient excretes more than 200 cc. of urine an hour. The urine is usually dilute, of lower specific gravity than the normal urine, and paler in color. It is possible in instances where 400 cc. of urine are excreted that the amount of pigment is no greater than would be present in 200 cc. of urine under normal conditions.

If it is thought desirable to overcome any error in quantitative estimation due to the presence of urinary pigment, one can make up a standard solution containing the same amount of urine as is obtained from the patients. The patient's own urine or any other specimen of the same color can be employed for this purpose. In this way very accurate quantitative estimations can be made. In the vast majority of cases, however, such correction is unnecessary, as it is not often that a patient voids more than 250 cc. an hour. In the majority of cases the technique of the test is simplicity itself. The injection is given and the urine is collected at the end of the first hour and at the end of the second hour. To each sample sufficient NaOH is added to insure alkalinity and maximum intensity of color; then this solution is diluted to 1 litre. A few cubic centimetres are filtered, the reading is made, and the percentage of drug excreted is calculated.

In a series of normal cases, studied in order to establish a standard, it was found that the time of appearance varied from five to eleven minutes, and that 40–60 per cent. of the drug was excreted in the first hour, and from 20–25 per cent. in the second hour, and that 60–85 per cent. was excreted in the two hours.

The excretion of the drug is not in proportion to the excretion of water. In many instances a high output of drug has occurred when the amount of urine was small, while in other cases the quantity of drug excreted was small, and the amount of urine was great. The smaller the amount of urine excreted in normal cases, the greater has been the concentration of the drug. It is immaterial, so far as the excretion of the drug is concerned, whether the urinary output is 50, 200, 400, or 500 cc.

The application of this test in various types of nephritis gave the following results:

In *acute nephritis* the functional ability of the kidneys fluctuates so much that in from 24 to 48 hours the results of the test may change from very good to very bad.

In cases of *parenchymatous nephritis*, with one exception, there has been a marked decrease in the amount excreted. In one case only 10 per cent. was excreted in two hours. The greatest decrease has been noted in cases where, clinically, marked secondary sclerotic changes were considered to be present. Indeed it is possible to estimate with a fair degree of accuracy the amount of interstitial changes. In the early mild cases the function may be but slightly disturbed, but in severe cases and cases of long standing it has frequently been found very low.

In *chronic interstitial nephritis* a low output was encountered in each instance, the decrease being usually proportionate to the degree of severity of the disease as estimated clinically. In two cases only a trace of the drug—less than 1 per cent.—was eliminated in the course of an hour. Both patients died of uræmia within two months.

The curve of elimination in nephritis differs from the normal in that the maximum intensity is reached slowly. This gives a slowly rising curve to the maximum, which is frequently not attained until the second hour. The excretion of the second hour is usually greater than that of the first.

In cases with *obstruction* in the lower urinary tract, almost all being patients with hypertrophy of the prostate, the elimination of the dye has been studied. These patients are frequently the subjects of pyelonephritis, pyonephrosis, pressure atrophy, and the resulting changes in functional activity. The urine output, urea, and total solids may be practically normal, and yet the patient be on the verge of a renal failure which will be precipitated by an operative interference.

The phthalein test has given valuable information in all these cases, differentiating those cases with severe renal damage from those in which the renal involvement was slight. As a rule the test has demonstrated the greatest impairment of function in such cases as have large residual urine and have not been leading a catheter life. Clinically, this type of case is recognized as the most dangerous when operation is undertaken without preliminary treatment. In many instances in which the output of drug was low when the patient was first seen, an adequate régime has resulted in a decided improvement of the kidney function, as indicated by the test.

When the time of appearance is delayed beyond twenty-five minutes,

and the output of drug is below 20 per cent. for the first hour, operation is postponed, regardless of the patient's clinical condition. If under routine treatment the output remains low but constant, the renal function is probably in a stable condition, and the operation may be performed, care being taken to select an anæsthetic which will not further depress the renal function.

When the residual urine is large, and the patient has not been leading a catheter life, even if the output at a single determination is large, operation is deferred, in order to determine whether the functional activity is stable; for it has long been recognized that following the relief of retention the function of the kidney is extremely variable. Repeated determinations should be made, and, except when unavoidable, operations should not be performed when the tests indicate a decreasing function. There have been two such cases in our series, in both of which operation was followed by death from acute suppression.

Again, when only a trace of dye is excreted operation should not be attempted, as grave renal changes exist. Two patients excreting only a trace died of uræmia within a short period. In neither case was any operation performed, though at the time of the first test no evidence of uræmia was clinically detected.

The test has made it possible to select for operation a time when the kidneys have regained their full functional power and stability. In no case in which the functional test indicated an efficient or stable renal function prior to operation has any evidence of renal insufficiency become apparent subsequently.

Rowntree and Geraghty proved also the value of the test in determining the functional value of the individual kidneys. The separated urines were obtained by ureteral catheters.

In normal cases the times of the appearance of the drug from the two sides have been almost the same—in the majority of cases from five to ten minutes. Frequently a slight difference of two or three minutes has been noted. In one case it appeared in six minutes on the left side, and in twenty-five minutes on the right. In this case, however, there was an anuria on the right side, probably reflex, but the collection of urine for one hour showed equal secretions of drug from the two sides. In only two normal cases has a distinct difference in the amount of drug excreted been observed.

Altogether seventeen cases of unilateral or bilateral renal infection were studied. Many of these cases came to operation, thus allowing opportunity to estimate the true value of the test.

*Unilateral Cases.*—When only one kidney is diseased, the appearance of the drug is delayed on the diseased side, and the amount excreted is not only relatively, but absolutely decreased. The amount

of delay in the time of appearance is comparatively of little value. Reliance is to be placed only on the quantity excreted during a period of at least one hour. It is possible by using large doses and extending the observations over a period of two hours, the excretion from each side being collected separately, to demonstrate in some degree the reserve functional ability of each kidney.

Although in the majority of these cases of unilateral disease the combined output is equal to that of two normal kidneys, the greater part of the excretion is shown to be accomplished by the healthy kidney. In approximate proportion to the decrease in function on the diseased side is the increase in function on the healthy side. In such cases following nephrectomy the remaining kidney eliminates an amount of drug which is normally excreted by two healthy kidneys. In all cases studied the output from the remaining kidney has been greater than the combined output from the two kidneys prior to operation.

**The Value of these Tests to the Surgeon.**—In the medical wards these tests may be said to add to the clinical pictures of cases, but for diagnosis not much weight is given them. For surgical conditions the case is very different, and these tests are of great value, when, *e.g.*, the question is the justification of removing a diseased kidney. In such a case the first question is, the presence of another kidney; the second is, can this second kidney do the work of both? To decide these questions, if by means of ureteral catheterization we can separate the urines excreted at the same time, a comparison of these is of value in determining, first, the presence of the second kidney; second, the relative values of their activity; while the freezing point of the blood will determine their united insufficiency; *i.e.*, as in medical cases, if lowered, a contraindication to operation. It is of interest that the confidence of the medical men in these tests has decreased, while that of the surgeons has increased.

The chief difference between these two points of view may be that surgical cases are chiefly of renal disease which destroys all renal function, while among the medical cases there are so many in which all the functions which can be tested are normal, but that unknown but all-important one, failure of which means uræmia, escapes detection. Kümmler<sup>172</sup> stated that he had in a long experience (in over 500 cases) never been deceived by cryoscopy of the blood, while Casper and Richer<sup>173</sup> consider the cryoscopy of the separated urines, together with the phlorizin test (determination of the amount of sugar eliminated by each kidney)—neither test alone but the agreement of both—of actually greater value than the microscopic or

<sup>172</sup> Centralbl. f. Chir., 1903, vol. xxx, II, p. 110; also Arch. f. klin. Chir., Bd. 67.

<sup>173</sup> Functional Diagnosis of Kidney Disease, 1903.

gross examination of the renal tissue. The surgeons have taken it for granted that the secretion of two normal kidneys at the same time is quite equal, although it is well known that the activity of a kidney varies much during successive intervals of time. But it is now known that for short intervals the simultaneous activity of the two kidneys is not equal. If collected during 15 minute periods the output of the two kidneys may differ 30 per cent.; if during 60 minute periods, 10 per cent.; and if collected during 10 hour periods the secretion of the two kidneys will be about equal. For this reason periods of at least two hours are recommended. The kidney can be said to be insufficient, when  $\Delta$  is less than  $-1^{\circ}$  C., unless the urine be diluted by recent intake of fluid.

The methylene blue test is of little value, since one cannot collect separated urines for a long enough time to determine the beginning, the end, and the intensity of secretion of this dye. Yet the surgeons do use it to determine whether the fluid from a sinus is urine or not. The phlorizin test is more valuable, since one can determine the time of onset, duration and intensity of the sugar excretion if he allows the catheters to remain in the ureters but three hours. But this is not necessary, since one determination of the sugar excreted by each kidney is a good index of the relative amount of renal activity of the two sides. The urine should be examined by the polariscope in from one-half to one hour after the injection. Barth,<sup>174</sup> who used the method of Casper and Richer, says that examination of the separated urines gives not an absolute, but a relative picture of the functional ability of the two kidneys.

Göbell<sup>175</sup> also determines only  $\Delta$ , and warns one that he cannot trust these functional tests implicitly. The patient should be for several days on a constant diet, the ureters catheterized at the same time after a meal, and the catheters left in place two to three hours to collect the urine. From  $\Delta$  one cannot tell whether or not the remaining kidney will be sufficient.

The dilution test has proved of very great, some say the most, value to the surgeon, the diseased kidney not responding as well as the other. The catheters are left in place for from three to five hours. The urine is first examined, the patient drinks 1.5 to 2 litres of water, and the voiding for each kidney is examined especially with regard to amount and  $\Delta$ .

The increased output on the normal side may begin during the second half-hour and reach a maximum in from two to three hours; the diseased side may show no increase.<sup>176</sup>

<sup>174</sup> Centralbl. f. Chir., 1903, vol. xxx, II, p. 134.

<sup>175</sup> Münch. med. Wochenschr., 1903.

<sup>176</sup> Illyés and Kovesi, Berl. klin. Wochenschr., 1902, p. 321.

Kümmel, on the other hand, considers the freezing point of the blood more important, and mentions 72 cases of nephrectomy without a mistake in judgment concerning the sufficiency of the second kidney, whether sound or slightly diseased. As a proof of the value of cryoscopy he states that before its use mortality of renal surgery was 28 per cent.; since its use, 8 per cent.; and of nephrectomy, 4.8 per cent.

Kümmel found in those cases in which there was an unconfirmed suspicion of renal trouble a constant  $\delta$  of  $0.56^\circ$ , with  $0.54^\circ$  to  $0.58^\circ$  as limits. (These figures all refer to depressions of the freezing point. When one says  $\delta = 0.56^\circ$  C. he means that the freezing point was depressed that much, that is, that the temperature of the freezing mixture was  $-0.56^\circ$  C., or  $t^\circ = -0.56^\circ$  C.) In the second group was disturbance of total renal function, bilateral nephritis, pyelonephritis, etc., with  $\delta = 0.60^\circ$  to  $0.65^\circ$ , limits  $0.59^\circ$  to  $0.81^\circ$ ; in these surgical cases of uræmia (prostatic cases *e.g.*) was a definite parallelism between the molecular concentration and the uræmic symptoms. In a long interesting series of cases of other than renal disease he finds  $\delta$  practically normal. He says that for him  $0.6^\circ$  is the limit of safety. If below this limit, although the one kidney may not be strictly normal, yet it is sufficiently so to do the work of both. The third group is a long series of cases of unilateral disease; when strictly unilateral, in all  $\delta$  was normal. In such cases ureteral catheterization showed on the affected side  $\Delta$  low and urea diminished, while the other side was normal. In cases of apparently unilateral disease with  $\delta$  normal, in no case did subsequent history show that there had been a bilateral disease. Cryoscopy may also be used in differential diagnosis; *e.g.*, if the question lies between renal calculus and hemorrhagic nephritis with unilateral pain, a high  $\delta$  would speak for the latter; in prostatic hypertrophy the presence or absence of an ascending disease can be settled by determining  $\delta$ ; that a tumor is renal can be suspected by the low  $\Delta$  of that side.

Recently it has been suggested that instead of methylene blue indigo-carmin, 3 cc. of a 4 per cent. solution injected subcutaneously, be used to test the functional ability of the kidneys. The advantages claimed for this dye are that it is excreted entirely by the kidneys, that its excretion begins in a few minutes and is soon over, and that no colorless excretion products are eliminated.

## CHAPTER III

### THE STOMACH CONTENTS

#### THE VOMITUS AND GASTRIC CONTENTS

THE various forms of vomiting have been grouped as follows:

Cerebral: in brain and cord disease, as tabes, insular sclerosis, meningitis of brain or cord, cerebral anæmia or hyperæmia, concussion of the brain, brain tumors, etc.

Toxic: opium, tobacco, ether, chloroform, alcohol, uræmia, cholæmia, pregnancy, *et al.*

Psychical: disgust, fright, anger, and other strong emotions.

Periodic: "cyclic," "recurrent," a form with sudden attacks of vomiting, often without apparent cause, and sometimes accompanied by intermittent hyperchlorhydria. There is evidence that some of these cases, especially of children, and which resemble a secretory neurosis, are due to an acidosis, *i.e.*, an autointoxication.<sup>1</sup>

Neurasthenia and hysteria: An interesting case of neurasthenia with very obstinate vomiting, as a rule, vomited repeatedly from three to four hours after the stomach was washed out. Each time she vomited from three to four ounces of bile-stained fluid.

Reflex: as in peritonitis, strangulation of the bowel, sexual disturbances, cholelithiasis, renal colic, intestinal worms.

Local: due to gastric conditions, whether acute or chronic, and especially those with stasis of the gastric contents.

**The Vomitus and General Considerations concerning the Gastric Contents.**—Considerable may be learned from vomitus, yet less than from a test meal. The gross inspection is valuable, its microscopical examination less so, and its chemical often misleading, for we seldom know the condition of the stomach previous to the meal which is vomited, nor always the character of this meal, at least we are sure it was of no standard quality and amount; the time element cannot be controlled, and it contains mucus and saliva from the mouth. Our test meal analyses, conducted with the greatest of care observing all of these points, are none too satisfactory, hence the examination of vomitus apart from its gross appearance is even less so.

The REACTION of vomitus, with the exception of a few cases of achylia, those with intestinal contents mixed, and a few cases of cancer of the stomach with alkaline gastric contents, is acid to litmus. If free hydrochloric acid is present it is an important point to exclude fluid from diverticula of the œsophagus.

<sup>1</sup> Edsall: Snow, Am. Jour. Med. Sci., 1904, vol. cxxviii.



The **character** of the vomitus is important; abundant, thin, acid fluid, with food eaten the previous day, means dilated stomach; very fluid, strongly acid juice free from food means continuous secretion; thin acid fluid with finely divided fragments of food suggests ulcer; thick masses with much mucus and poorly digested often decomposing meat suggest chronic catarrh and cancer of the stomach; recently eaten, undigested food suggests nervous vomiting. If the vomiting occurs at the height of digestion and during a paroxysm of pain which then at once diminishes, one thinks of ulcer; if during or shortly after eating, of cancer, catarrh, or a neurosis; if independently of eating, often mornings before breakfast, and it contains not only mucus and bile but also food remnants, of ectasis. Cerebral vomiting is often marked by a noticeable absence of straining or effort; vomiting on rising in the morning is suggestive of pregnancy, or, in the case of men, of alcoholism; with cancer at the cardiac orifice vomiting follows a meal; if there be pyloric stenosis due to any other cause the vomiting occurs later, at longer intervals, and in large quantities.

A very slight BLOOD streaking of vomitus and gastric contents is of no moment, since from the effort of vomiting or by the stomach-tube slight lesions of the œsophagus or pharynx may result.

**BILE AND PANCREATIC SECRETION.**—Traces of bile are often present in vomitus from a fasting stomach, at the end of lavage, and in vomiting attended by severe retching. This has no significance unless it be constantly present and there has been no straining sufficient to force bile from the duodenum into the stomach. In case it is constantly present, it might indicate stricture of the duodenum below the ampulla. On the other hand, a green color does not always indicate bile, since a few cases are recorded<sup>2</sup> of "grass green," "sea green," "dark green" vomitus, the color of which is due to algæ or at least to chlorophyll-colored protophytes. Bile-stained vomitus is particularly common from an almost empty stomach, less so from a full one, in which case there is more counterpressure against the pylorus, preventing the regurgitation of bile. For this reason it was thought the vomitus of peritonitis was more often bile-stained than of cerebral troubles, since in the former cases the stomach is more often empty.

Mucus in the vomitus is almost constant. Seldom, however, does it indicate gastric catarrh, a condition which is rare compared with the number of times that the diagnosis is made. It may be due to catarrh, but is more often to lack of hydrochloric acid and hence lack of digestion of the mucus that is normally secreted. The morning vomitus of alcoholics contains large amounts of mucus.

Large amounts of acid gastric juice, sometimes pure, sometimes mixed with food, are common in cases of hypersecretion, the former

<sup>2</sup> See Kuhm, *Zeitschr. f. inn. Med.*, 1902, No. 28; 1903, No. 1.

especially in cases of gastroxynsis, a neurosis with periodic attacks of acid vomiting. If food be present in this case the proteid will be well digested, the starch less so.

The vomiting of large amounts in which is food eaten two or three days previously occurs in cases of stricture of the pylorus with dilatation of the stomach. In this vomitus the proteid will be poorly digested and badly fermented in case of cancer, etc., well digested with fermentation of carbohydrates in benign cases due to ulcer, etc., this depending on the presence or absence of hydrochloric acid.

FECAL VOMITING occurs when there is complete obstruction of the ileum or the colon, or paralysis of the intestinal wall due to peritonitis, etc. The patient vomits repeatedly and each vomitus is more fecal than the others, so that it is easy to say approximately from what part of the intestine each comes. Finally the vomitus is the black, foul-smelling contents of the colon, which microscopically contains vast numbers of bacteria. Yet the absence of fecal vomiting does not always exclude a total obstruction, as when it is high in the jejunum. For the vomitus to have even a suggestive fecal odor the obstruction must be at least six feet from the pylorus.

RICE-WATER VOMITUS is seen in Asiatic cholera. It is very fluid and filled with white flakes of mucous shreds and epithelial cells (see page 388).

From the *color* and the *odor* of the vomitus cases of poisoning or alcoholism may be suspected. In uræmic cases it has an ammoniacal odor.

Some idea of the *motility* of the stomach may also be obtained, since if any food is vomited seven hours or more after the last meal, motility is certainly diminished, although this may be a very temporary condition. At the end of two or three hours particles of meat should be swollen and show considerable evidence of digestion. At the end of one hour bread should have been broken up to a fine, crumbly sediment, which settles to the bottom of the glass. If there are large particles of bread, and especially if these are coated with mucus, hydrochloric acid is quite surely diminished.

The **chemical analysis** of vomitus, as stated above, is exceedingly unsatisfactory. If free hydrochloric acid be present we are sure that it is secreted, but, if absent, we can draw no conclusions. In general, it may be said that normally both free hydrochloric acid and pepsin are present two hours after a mixed meal.

Lactic acid may be expected after a mixed meal.

The **microscopical examination** is also unsatisfactory, since normally both intact muscle-fibres and starch granules pass to the intestine.

The *antiseptic condition* of the stomach may also be judged. The presence of organisms may be due either to the absence of hydrochloric

acid or to stasis. The vomitus may be foamy and have the odor of butyric or other organic acids. In cases with free hydrochloric acid and severe stasis the majority of organisms are yeasts and sarcinae; in lighter cases of stasis the fluid is sterile; in those cases, as of cancer, without free hydrochloric acid, bacteria predominate.

TUMOR FRAGMENTS OCCUR, but are rarely found. In the vomitus may be found round worms, segments of tape-worm, oxyuris, maggots, etc.

**Examination of the Fasting Stomach.**—While from the normal fasting stomach theoretically no fluid should be obtained through the stomach tube, yet it is very common to get from 10 to 50 cc. of an acid gastric juice. While there is some difference of opinion as to what amount should be considered the upper limit of normal, we have agreed with Boas that 100 cc. or more may safely be considered abnormal and would indicate hypersecretion or motor insufficiency. These two conditions may be differentiated by washing the stomach out at night; if there is motor insufficiency, the stomach will be empty in the morning. Riegel insists that the normal fasting stomach is always empty, and that to find even a little fluid is pathological.

THE FLUID FROM THE FASTING STOMACH is thin, its specific gravity from 1004 to 1005, it contains some free hydrochloric acid, no lactic acid, and no bacteria. In many cases it is bile-stained, but this is not important unless it is found so on several examinations, in which case duodenal stricture may be suspected. If alkaline from the presence of pancreatic juice, trypsin may be tested for. To find trypsin in an acid or neutral fluid soda must be added at once to prevent the destruction of this ferment. Abnormal amounts of mucus may be present as in anacidity, atrophy of the mucous membrane, etc., but considerable washing is necessary to dislodge much mucus from the mucosa.

**Test Meals.**—THE EWALD-BOAS TEST BREAKFAST consists of white bread, about 40 gms., water or tea without sugar or cream, about 400 cc. The bread should be chewed very fine. This breakfast is to be removed in just one hour. Usually 30 to 70 cc. are obtained, of specific gravity 1012 to 1020. If 200 to 300 cc. are gained, there is hypersecretion, motor insufficiency, or perhaps disturbed absorption.

RIEGEL'S meal consists of one plate of beef soup, from 150 to 200 gms. of beefsteak, and 150 gms. of mashed potatoes. It is to be removed in from three to four hours.

Riegel and others have emphasized that a test meal should be one to which the patient is accustomed. For this reason, in Germany the breakfast consists of bread and tea or water, and the test meal of beef, etc., since this is a fair sample of the diet to which the German laborer is used. Both Ewald and Riegel also insist that the meal should be

given at that time of the day at which the patient is accustomed to ingest a meal of that character. But in this country such meals, particularly the Ewald breakfast, are not customary, are not in the least like ours, and yet American observers quite uniformly use this breakfast, overlooking the fact that one cannot test normal physiological phenomena with abnormal meals.

Again, meals should be chosen with reference to the case. Not only does the average fare differ in different countries and in different grades of society, but the important individual peculiarities of taste and habit cannot be totally disregarded. In adopting these two German meals we therefore entirely neglect the two important points,—that the meal should be one to which the patient is accustomed, and given at the hour at which he is accustomed to take it. It is small wonder that many are sceptical as to the value of their use. But the work of physiologists (Pawlow) has shown that in the secretion of gastric juice the psychical element—that is, the influence of taste, sight and smell, etc.—is almost equal to the chemical element,—that is, the stimulus from the absorption of soluble products of gastric digestion,—and since the Ewald breakfast is certainly unpalatable, it is perhaps valuable for that reason, since it rules out in some measure the former factor, and while to find diminished acidity may not mean much, yet hyperacidity does mean that there is certainly some trouble present. Many Americans appreciating these facts have attempted to introduce meals for American patients. Of course, no two persons' tastes are alike, yet we can select as standard a meal more like our patients' diet than the above. FISCHER'S MEAL is perhaps as good as any, which consists of the bread and water of the Ewald breakfast plus a quarter of a pound of finely chopped lean beef broiled and slightly seasoned. It is to be removed in three hours.

Fischer has shown by comparing results with his meal with those of the Ewald breakfast that those with his are much more constant than with the latter. For instance, repeated examinations were made with the Ewald breakfast; in 40 per cent. of the cases the findings with the later meals were quite different from those with the earlier. With his meal there was need of changing the diagnosis in but about 8 per cent. of the cases. Using both meals in the same cases, in 67 per cent. they gave similar results, 18 per cent. of those showing hyperacidity with the Ewald meal showed less with his meal, and in 15 per cent. more. Of the subacidity cases with the Ewald, 30 per cent. were normal by his. In various clinics, we have noted that several meals were used, the Ewald meal giving some idea of what the stomach will do when an indifferent meal is given which excites but little secretion, the Riegel indicating the possibilities when there is greater tax upon the secretory cells. Some can handle the test breakfast well, but cannot the larger meal, while others respond well only to the greater stimulus. Fischer gives several points which might aid in differential diagnosis based on the use of two meals. Concerning the diagnosis of the anatomical lesion he says that if the stomach be subacid to the breakfast but normal after a proteid meal, we can state that the secretory structures are normal, and may suspect that atony with the constant

presence of food has rendered the mucosa less sensitive; if subacid to the proteid meal as well as to the breakfast, it indicates organic changes; if subacid to the breakfast but hyperacid with a proteid meal, it could mean defective innervation; the same is true if hyperacid to the breakfast and normal to the larger meal; if the hyperacidity of the breakfast continues and increases with the proteid meal one may suspect a probable increase of oxyntic cells, since the secretion is not in proportion to the stimulus, especially if the secretion continues several hours after the meal. If the symptoms and the increased secretion both diminish after the meal, disturbed innervation may be suspected. He also emphasizes the fact that certain cases of dyspepsia which have been on an almost starvation diet for some time need to be fed up pretty well before a test meal is given. This may cause a gastric upset, but the flare-up of the condition will be an advantage in the diagnosis.

In all cases we should be sure that the stomach is empty before the meal is given. This may be done in the case of the breakfast by lavage the preceding night, unless experience teaches us that there is in this case no motor insufficiency. The patient should get used to the meal and to the tube, and hence the first meal is seldom of any value, and the second should be confirmed by at least one other. Another point on which sufficient emphasis has never been laid is that the meal should be removed at the time of optimum secretion. The Ewald test breakfast should not always be removed at the end of sixty minutes, nor the Riegel at the end of five hours. With the Ewald breakfast, as is easily seen by the study of cases in which meals are removed at different intervals, in some cases with rapid motility the maximum acidity is attained in forty minutes, in others in an hour and a quarter. In either case if the meal be removed at the end of just an hour, an erroneous idea will be gained by the low acidities found. Another point particularly important in neurotic cases is that the time at which the meal is given should be chosen with reference to the symptoms, since at other times than during the nervous disturbances the gastric condition may be normal.

Many other meals have been proposed, *e.g.*, the whites of two hard-boiled eggs and 100 cc. of water (Jaworski and Gluzinski), and much more complicated ones (Pfaundler-Sahli, *et al.*). We have tried nutrose to some extent, in the hope that the pure proteid meal would teach more concerning the products of proteid digestion. This meal is not at all appetizing, and was given up.

While it has long been recognized that we were foolishly fitting to the American stomach European meals, and need new standards following the use of American meals, yet in a general way much has been learned from these two meals.

**Acidity of the Gastric Juice.**—The fluid removed after the test meal or breakfast should be first tested with litmus. This will indicate acidity in general, which may be due to hydrochloric acid free or bound, to organic acids should they be present, and to acid salts. In the great majority of cases the litmus will show acid; in a very

few cases the fluid is alkaline. In a recent case of cancer in these wards the fresh fluid was alkaline to litmus. It contained many pus-cells. The next point is the presence of free acid, and Congo red is the indicator usually used, indicating free organic or inorganic acid.

The tests for FREE HYDROCHLORIC ACID are all of them color-tests. The first and perhaps the commonest used, since it is the easiest, is the above-mentioned Congo-red paper. Free hydrochloric acid will turn this to a sharp blue, while free organic acids, even in strong concentration, will give a much less definite blue. A trained eye usually has no difficulty in recognizing from this test alone whether it be free hydrochloric acid or free lactic acid that is present. Acid salts, if strong, give a positive test with Congo-red, but they do not occur in this concentration in the stomach.

Methyl violet is the indicator first suggested by V. d. Velden, who first showed the presence of free hydrochloric acid in the gastric juice.<sup>3</sup> This is a very satisfactory test. One drop of the saturated aqueous or alcoholic solution of methyl violet is mixed with water in a test-tube until the color is a pale violet. This is divided in two test-tubes; to the one is added the filtered gastric juice, to the other the same amount of water. Free hydrochloric acid will turn the violet to a fine blue color. It requires a much larger amount of organic acid. This indicates 0.025 per cent. free hydrochloric acid.

Tropæolin OO has been used, but is less sensitive than the above, indicating 0.03 per cent. In this case the saturated solution is used in the same way as the methyl violet. If free hydrochloric acid is present, the yellow is turned to a reddish-yellow color. Boas suggests that this be used as a contact test, a few drops of the concentrated solution of the stain being warmed in a porcelain dish and brought into contact with a small amount of the gastric juice. The dish is then warmed over a small flame, and if free hydrochloric acid be present a fine violet or true blue color is formed.

Dimethylamidoazobenzol is most commonly used, and is a very sensitive indicator. It gives a pink color with free hydrochloric acid, but it reacts also to organic acids and acid phosphates in concentrations which might occur in the stomach.

Günzberg's solution (Phloroglucin, 2; vanillin, 1; alcohol, 30) is the standard test. One or two drops of this solution (which should be kept in a tightly corked blue bottle) are warmed on a porcelain dish until just dry. One drop of the gastric juice is then allowed to come into contact with this and the warming continued. If free acid is present, at the edge of contact will appear a beautiful crimson line. This was considered very sensitive, showing 0.005 per cent. of the free hydrochloric acid. Now, some (*e.g.*, Unterberg) says it is less sensitive than others. It is of value, since it indicates nothing but free inorganic acids. The test, although so easy, is often spoiled, since the solution is burned by too much heating and a brown non-characteristic color appears. The fluid should not be allowed to get too old.

<sup>3</sup> Deutsch. Arch. f. klin. Med., Bd. 23.

It is to be emphasized that the above color-tests are for free hydrochloric acid; that is, for the acid in excess of all acid binding bodies, such as proteids, hexone bases, etc.

Free acid is present in the stomach after a carbohydrate meal in from one-half to three-quarters of an hour; after meat, from one to one and one-half hours; and after milk and potatoes in three-quarters of an hour.

**Total Acidity.**—This is the starting-point in all gastric analyses. This figure represents the highest amount of hydrochloric acid which could possibly be present, and, compared with the amount of free acid, gives a good picture of the secretory function and the motility of the stomach.

Acid bodies which could be present are the free and the bound hydrochloric acid, other acids, as lactic, butyric, etc., and acid salts.

To 10 cc. of filtered gastric juice is added an indicator. Tenth-normal NaOH is then added from a burette, stirring the fluid all the time until the first change of color is seen throughout the whole volume. This titration may be done in a porcelain dish, a beaker, or an Erlenmeyer flask, the latter two against a white background.

The indicator usually used is phenolphthalein, two or three drops of a 0.5 per cent. alcohol solution. Others have been proposed, among them litmus, cochineal, methyl orange. Phenolphthalein is preferred because of the sharpness of its reaction, yet it is perhaps the worst indicator possible, since this sharp end reaction does not always indicate the point of neutralization of all the acid elements, especially since ammonia is sometimes present in no small amount. The reason for its continued use is the desire for comparable results to which an empirical value may be given. The same gastric juice will give very different results with different indicators, and those with this are too high.

Some use the filtered gastric juice, some the unfiltered, shaking it up well to a homogeneous suspension, since the solid particles contain relatively more of the acid than the fluid portions.

Two methods of expression of results are in vogue; the one to estimate from the amount of sodium hydroxide used the equivalent amount of hydrochloric acid. The number of cubic centimetres of the sodium hydroxide used multiplied by 0.00365 gm. equals the amount of this acid by weight in 10 cc. In the case of total acidity this would be the highest possible amount of hydrochloric acid which could be present, hence has the advantage of stating the outer limit of possibility, although a certain amount of total acidity is surely not due to hydrochloric acid. As an exact statement of truth the suggestion of Jaworski is usually followed; that is, the number of cubic centimetres of the alkali which would be required to neutralize 100 cc. of the

gastric juice, and to this the term "acidity per cent." is applied. Since 10 cc. are usually used, the titration figure multiplied by 10 will give the acidity per cent. without reference to the acids which may be present.

As illustration: if, using phenolphthalein as indicator, 10 cc. of the juice required 8 cc. of tenth-normal NaOH to neutralize the acids present, the acidity per cent. would be 80. Supposing that HCl were the only acid present, then the gastric juice would contain 0.29 per cent. HCl. To avoid confusion, the symbol of percentage is never used for "acidity per cent."

TABLE OF EQUIVALENTS.

Acidity per Cent.	Gravimetric per Cent.
10 .....	0.0365
14 .....	0.05
20 .....	0.073
27 .....	0.1
34 .....	0.125
40 .....	0.146
48 .....	0.175
50 .....	0.182
55 .....	0.2
61 .....	0.225
70 .....	0.25
73 .....	0.275
80 .....	0.292
87 .....	0.317
90 .....	0.329
95 .....	0.347
100 .....	0.365
105 .....	0.383
109 .....	0.4

**Free Hydrochloric Acid, MINTZ METHOD.**—Ten cc. of the filtered gastric juice are titrated with tenth-normal NaOH until the test for free acid is no longer positive. This is based on the supposition that the NaOH will neutralize the free before the bound HCl.

Of indicators there are several. Undoubtedly the most accurate is the Günzberg. As the sodium hydroxide is added, small drops of the stirred fluid are removed by a glass rod, or, better still, a platinum oesa, and tested on a porcelain dish (see page 356). Fleiner adds 25 to 30 drops of the Günzberg reagent directly to the gastric juice, and then, as the sodium hydroxide is added, removes small drops which he warms in a porcelain spoon. Sahli recommends that the glass rods themselves with which the soda is stirred into the gastric juice be warmed, since the crimson color can be seen on the rod. He also adds from 25 to 30 drops directly to the fluid. In this method a certain amount of gastric juice is lost in each of the tests, and hence the results should be confirmed by a new portion from which less is removed.

A much easier method, and one that is chiefly used in some clinics



in which the best gastric work is done, is to add the sodium hydroxide until small drops touched by a rod to Congo-red paper no longer turn this blue. Some find approximately the end reaction with Congo-red, and then more definitely with the Günzberg. The Congo-red should be used as a paper moistened by drops removed from the fluid, rather than added as a solution to the fluid, since with free acid results a suspension of a bluish-black precipitate which makes the end reaction difficult. As little should be removed as possible for each test, and the color produced by the drop controlled by one with distilled water.

*Töpfer Method.*—The method in quite common use in this country, since it is the quickest, employs dimethylamidoazobenzol as the indicator. The smallest drop possible, in fact, a small fraction of a drop from the end of a glass rod, is added to the gastric juice, which will take a bright red color. The sodium hydroxide is now added until the red element of the color is lost. The end reaction requires a trained eye, since the transition from bright red to clear yellow is a broad one, with the important point the disappearance of the red shade.

The amounts of free acid determined by these three indicators are by no means the same, as the difference sometimes amounts to 100 per cent. Günzberg will always be the lowest and dimethylamidoazobenzol usually the highest. Congo-red paper varies very much, some qualities giving results almost as low as Günzberg, some the highest of all.<sup>4</sup>

**Hydrochloric Acid Deficit.**—Tenth-normal HCl is added to 10 cc. of the gastric juice until the test for free acid is positive. The amount necessary will depend on the amount of bound HCl already present, the amount of proteid and bases to bind the acid, and the amount of alkali secreted, hence a better term suggested by Sahli is the "saturation deficit." Congo-red paper or Günzberg can be used, but the former is sufficiently delicate. The determination of the HCl deficit is quite as important as of the free acid, since the progress of the case either downward or toward improvement can thus be followed.

For the bound hydrochloric acid Töpfer recommends alizarin as indicator. This now is little used.

Fischer,<sup>5</sup> after neutralizing with tenth-normal NaOH for total acidity, then adds an amount of tenth-normal HCl equal to that of the alkali added, and then a 4 per cent. calcium phosphotungstate solution to 30 cc. It is allowed to stand three to four minutes, animal charcoal added, and filtered. To a measured part of the filtrate 6 drops of 1 per cent. rosolic acid are added, stopping at a deep orange tint, and its acidity determined. All the proteid has thus been precipitated, and the bound HCl left as  $\text{CaCl}_2$ . The total acidity minus that of the filtrate equals the combined acid. In case no free acid is present, the deficit is

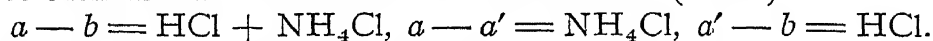
<sup>4</sup> See Johns Hopkins Hosp. Bull., January, 1903.

<sup>5</sup> Am. Jour. Med. Sci., 1903, vol. cxxvi.

first determined, then the total acidity. Enough tenth-normal HCl is then added to raise the total acidity at least 40 per cent., then one precipitates the proteid with calcium phosphotungstate and proceeds as above. A simple calculation gives the bound acid.

**Total Hydrochloric Acid.**—Hydrochloric acid is present free, bound, and as neutral chlorides. Of the latter we have those from the food, those formed in the stomach, and those secreted as such.

By the total hydrochloric acid is understood the bound and the free ("the physiologically active hydrochloric acid"). The Lütke-Martius method is one of the simplest for determining this. The principle on which this is based is that the difference between the total chlorine ( $=a$ ) and the chlorine after incineration ( $=b$ ) represents that volatilized by heat; *i.e.*, the hydrochloric acid. This method has been corrected by Reissner, who showed that with the HCl,  $\text{NH}_4\text{Cl}$  is also volatilized. He, therefore, first neutralizes the gastric juice with tenth-normal NaOH, using litmus as indicator. This neutralized fluid is then ashed and the chlorine determined ( $=a'$ ).



The Arnold and Lütke methods are used to determine chlorides. (For the solutions necessary, see page 131.)

*Determination of "a."*—Ten cc. of the gastric fluid are measured with pipette into a flask with a 100 cc. mark on its neck. Twenty cc. of Solution 1 are then added, stirred, and allowed to rest for ten minutes. A few drops of 8 per cent.  $\text{KMnO}_4$  are then added if necessary to decolorize. The flask is then filled with water to the 100 cc. point and the contents well mixed. The fluid is then filtered through a dry filter until one-half has passed through. Fifty cc. of the filtrate are measured into a beaker and Solution 2 added from a burette till the first permanent brown.

The number of cubic centimetres of Solution 2 necessary to precipitate the excess of silver are then multiplied by 2, since but half the fluid was used in this titration. This product, subtracted from the amount of  $\text{AgNO}_3$  originally added, will give the amount of  $\text{AgNO}_3$  used in precipitating the chlorine.

*Determination of "b."*—Ten cc. of the gastric juice are evaporated to dryness on a water-bath in a platinum dish. This is then burned over the free flame until the ash no longer burns with a luminous flame. It is not brought to a red heat, since this would volatilize some of the chlorides. The ash is then rubbed up well with water by a glass rod, extracted with about 100 cc. of warm water, brought onto the filter and washed until a few drops of the filtrate no longer give a precipitate with  $\text{AgNO}_3$ . To the whole filtrate are then added ten cc. of Solution 1, and the determination proceeds as for "a."

*Determination of "a'."*—Other ten cc. are first neutralized with tenth-normal NaOH, using litmus as indicator, then ashed and the remaining chlorides determined as for "b."

$(a' - b)$  0.0365 gm. = the per cent. of HCl.

**Töpfer's Method.**—This method promised to be a very simple and useful one, since it was purely volumetric and could be finished in a very few minutes. The stomach contents were titrated with dimethyl-amidoazobenzol for the free hydrochloric acid, and with a 1 per cent. aqueous solution of alizarin for the bound hydrochloric acid. This

method received severe criticism at once, since both indicators were not above criticism and the end reactions rather doubtful, yet Hari in Boas's laboratory found it quite as accurate as some of the more elaborate methods if free hydrochloric acid is present; if absent, inaccurate.

**Absolute Amount of Hydrochloric Acid Secreted.**—It is of course evident that the preceding methods give merely percentage values, the percentage of the acid in the stomach contents at that time, and without reference to the total amount of acid at other times present. The absolute amount of hydrochloric acid at a stated time has been a matter of considerable investigation, especially by Bourget and Geigel.

The last method proposed is the following, the preceding methods being all rather tedious. The stomach-tube is introduced and as much of the gastric juice expressed as possible. Then 300 cc. of water are allowed to flow in and out of the stomach several times. From the difference in the specific gravity of these two fractions the amount of undiluted gastric juice in the second fraction can be computed. The acid of the first fraction is then determined and that of the second calculated and added to it. This method has some scientific, but no practical value, since considerable of the acid secreted has already passed into the intestine.

Leo's method for **total acidity** is based on the principle that all acids whether free or bound are neutralized by  $\text{CaCO}_3$  while acid salts and acid binding bodies remain unchanged. To 10 cc. of the gastric juice are added 5 cc. of concentric  $\text{CaCl}_2$  solution (since acid phosphates may be present), and titrated with tenth-normal  $\text{NaOH}$ , using phenolphthalein as the indicator. Let the number of cubic centimetres used be "*a*."

Fifteen cc. of gastric juice are rubbed up with 1 gm. of pulverized and dry  $\text{CaCO}_3$ , and filtered through an ash-free filter. Of the filtrate, 10 cc. are freed from  $\text{CO}_2$  by a stream of air, and then 5 cc. of  $\text{CaCl}_2$  solution added, and this fluid titrated as above (result = *b*).

*a* — *b* equals the total acid. If no organic acid be present, this is all  $\text{HCl}$ .

**The Value of the Tests for Acidity of the Stomach.**—Among the above tests the simple presence or absence of free hydrochloric acid is of the greatest importance, much more so than is its percentage amount. Practically every stomach can secrete some acid, but the normal stomach will always secrete a physiological excess. It may be that the bound acid is alone of value in digestion of proteid, but it is the amount free which is the index of the functional ability. The percentage of the free acid is of importance in determining the question and grade of hyperacidity, and the deficit to determine the grade of the case and to follow it in its improvement. It is to be remembered that accurate quantitative work requiring considerable time is not justified, for only percentages can be determined, since the total amount can never be obtained. The point of departure is the determination of total acidity. If the Ewald test breakfast is used the phosphates are unimportant, and hence the acidity is the sum of the hydrochloric and organic acids. These are never present together free and if free hydrochloric is present the organic acids can be disregarded. Knowing these few points, valuable deductions can be drawn. For instance (quoting Sahli), if the total acidity be high and no free hydrochloric

present, the acidity is due for the most part to free organic acids. If the lactic acid test be good or the odor of the other organic acids perceived and many bacteria are present, this diagnosis is confirmed. If free hydrochloric is present and the total acidity low, the most will be due to hydrochloric acid, and the motility of the stomach may be assumed to be good, since the acid binding bodies have passed on into the duodenum. If, on the other hand, the total acidity be moderate, and free acid small in amount, a poor motility may be assumed, with the retention of the products of digestion.

**Physiology of the Gastric Juice.**—After the ingestion of a test meal secretion of gastric juice begins almost at once. All the hydrochloric acid is at first bound. By the end of a half-hour after the test breakfast or two hours after the Riegel meal, as a rule, enough has been secreted so that some remains free. The amount of acid rises to a maximum, and then as the products of digestion pass on into the intestine the acidity begins to fall. One hour after the Ewald breakfast the total acidity averages normally 40 to 60 acidity per cent., or 0.15 to 0.22 per cent. HCl; over 0.25 per cent. means hyperacidity (some say 0.2 per cent.). Lactic acid is not present. Acid phosphates are no factor. The free HCl is from 20 to 60 acidity per cent. or 0.05 to 0.2 per cent.; the bound from 0.012 to 0.11 per cent. Although as a rule there is most in the stomach one hour after the meal, yet this will depend on the motility of the stomach. In some cases the time to remove the meal is forty minutes and in other cases at one and a quarter hours from the time the meal was eaten. It is important, therefore, to determine the optimum of each case before drawing any conclusions.

With the Riegel meal the normal total acidity is about 75, but from 90 to 100 acidity, and free about 44 may be present.

**Diagnostic Value.**—Sahli gives the following very valuable summary:

A. There is normal acid secretion: (1) Often in ulcer of the stomach and stenosis due to the contraction of its scar; (2) gastric neuroses; and (3) simple atony.

B. Hydrochloric acid is increased, that is, the free is more than 0.2 per cent. and the total is more than 70 acidity per cent. (it may reach 0.35 per cent. and very rarely 0.8 per cent.): (1) In the majority of cases of ulcer of the stomach; (2) in true continuous hypersecretion (but not the hypersecretion due to motor stasis); (3) simple hyperacidity and hypersecretion occur only during digestion, at which time the per cent. of acid is abnormally high; (4) paroxysmal hypersecretion (gastroxynsis) occurring in neurotic individuals, who, following some excitement or other disturbance, vomit large amounts of acid juice; (5) in some cases of chlorosis (in 22 of 30 of Riegel's cases); (6) early

stages of chronic gastric catarrh; (7) often in insanity.

C. The hydrochloric acid secretion is diminished in (1) fevers; (2) severe anæmias; (3) the majority of cases of chronic gastric catarrh; (4) many gastric disorders due to general neuroses; (5) many forms of mental disease; (6) after long standing jaundice; (7) many chronic cachexias, as tuberculosis of the lung, but not always; (8) chronic passive congestion due to heart disease or to emphysema, etc.; (9) sometimes in chronic nephritis; (10) after the long use of alkaline and saline purges; (11) as a "fatigue" symptom following periods of hypersecretion.\*

D. Free hydrochloric acid is absent on several examinations (and yet the stomach always contains a certain amount of this acid bound) in all conditions under C of a severe grade; such as amyloid disease of the stomach, toxic gastritis, nervous dyspepsia, phthisis, cardiac disease; especially in (1) severe febrile diseases, particularly the infections; (2) gastric carcinoma (also other carcinomata); (3) atrophic gastric catarrh; (4) pernicious anæmia. The most important is the failure of free hydrochloric acid in gastric carcinoma.

And yet in gastric analysis the figures are simply relative to the case, for some patients are distressed by acidities normal to other persons. The absence of free hydrochloric acid is always abnormal.

Standards vary with nationalities, especially with classes of society and still more with individuals. Strauss, at Giessen, thought 68 a fair average total acidity; at Berlin, 47. In this country we must not try to make German acidities fit any more than the meals producing them; and if possible, every case should be considered individually.

Among 526 cases of this clinic whose records were studied are the following. In all cases the Ewald breakfast was used.

PERNICIOUS ANÆMIA, 13 cases. Amount removed, 10 to 80 cc. All were subacid, the highest total acidity being 38 (acidity per cent.) and below 10 in 10 cases. In only one was any free hydrochloric present. In two the fluid was neutral to litmus; in one alkaline. Lactic acid was present in two cases (in one there was an autopsy, in the other not). In two cases of severe *secondary anæmia* the fluid was only slightly subacid, and free hydrochloric acid was present.

MALIGNANT DISEASE NOT OF THE STOMACH.—Of these, ten were carcinomata, four sarcomata. All were subacid (total acidity less than 40). Of the carcinoma cases, free hydrochloric was present in seven, absent in two, and the fluid neutral to litmus in one. Of the sarcoma cases, in none of the four was free hydrochloric present.

CATARRHAL JAUNDICE, 9 cases. The fluid removed varied from 10 to 86 cc.; total acidity, 10 to 70; in three cases no free hydrochloric acid; in one the fluid was alkaline to litmus; in none was lactic acid found. In a few cases the acidity progressively diminished during the course of the disease.

CHOLELITHIASIS, 14 cases. Amount removed 5 to 120 cc. There was hyperacidity in one case (total 79 and 82; free HCl, 42 and 49 respectively on two examinations). Normal acidity (40 to 70) in six cases; below 40 in six; and in four of these no free hydrochloric; lactic acid in one.

CIRRHOSIS OF LIVER, 6 cases. Normal acidity obtained in one, subacidity in five,

\* Foster and Lambert, The Jour. of Exp. Med., 1908, vol. x, No. 6, p. 820.

in four of the six cases there was no free hydrochloric acid, in one the gastric juice was practically neutral, while in one there was lactic acid.

TUBERCULOSIS OF LUNGS, 10 cases. Normal total acidity in three; subacidity in seven; no free acid in two. Tuberculosis of other organs, five cases; normal acidity in one; no free acid in three; in one of these the fluid was neutral; in one lactic acid was present.

INTESTINAL TROUBLES.—*Diarrhœa*, 9 cases; in four the acidity was normal; in four subacid, in one almost neutral. In four no free acid was present; in one there was lactic acid. *Constipation*, 5 cases, of which two were normal, three subacid, and one without free hydrochloric *Colitis*, 2, both subacid and without free acid, and both with lactic acid. *Amœbic dysentery*, one hyperacid (92 total and 83 free).

ARTERIOSCLEROSIS AND CARDIAC DISEASES, 17 cases. Eight normal, six subnormal, three without free acid, and one almost neutral.

In a group of 36 cases of MISCELLANEOUS DISEASES, twenty-five showed normal gastric conditions. Subacidity without free acid was present in cases of heat prostration, enteroptosis, chronic bronchitis, peripheral neuritis (with lactic acid), chronic nephritis, bronchopneumonia, and malaria.

#### SAHLI'S DESMOID REACTION.\*

This method is based on the assumption that catgut in its raw state is soluble in the gastric juice, but is entirely indigestible in the pancreatic juice.

One forms a pill of methylene blue, 0.05 gram, or of iodoform, 0.1 gram, or of both, together with sufficient ext. glycyrrhizæ to make the pill not over 3 or 4 mm. in diameter, and encloses this in a rubber sack, which is done by twisting the pill in the centre of a square piece of thin rubber dam and tying its twisted neck with 3 turns of number 00 raw catgut previously soaked in cold water until soft, care being taken that the knots are both on the same side of the bag. The rubber is then trimmed away carefully, so that only a little free edge, about 3 mm., remains beyond the ligature. It is important that the cut edges of the rubber should not cohere, that the complete pill should sink instantly in water, that it should be water-tight, and that the rubber should not be drawn so tightly over the pill as to become permeable to water.

This "desmoid pill" is given with or just after the midday meal, and the urine is collected at periods of 5, 7, and 18–20 hours later and examined for methylene blue or iodine or both. The methylene blue is recognized by the greenish-blue color imparted to the urine, or, in the absence of this color, by the greenish-blue color which appears when the urine is boiled with one-fifth volume of glacial acetic acid if only the chromogen of methylene blue is present. It is very rarely indeed that the chromogen alone is excreted; in nearly all instances in which the urine when voided is colorless the gut has not dissolved, and so the bag has not opened. The iodine is

\* Sahlî, Corresp. Bl. f. Schweiz. Aertzte, 1905; Boggs, Johns Hopkins Bull, 1906, vol. xvii, p. 313; Carey, Boston Med. and Surg. Jour., May 2, 1907, vol. clvi, p. 562.

recognized by the rose color that is observed when the urine is strongly acidified with pure nitric or sulphuric acid, and then shaken out with a little chloroform. It may be tested also in the saliva with starch paper, or as above. If the methylene blue or iodine be found within the 18–20 hours after the ingestion of the pill, the test is called positive; otherwise, negative.

The advantages claimed for the test are that it is simple in application, that it causes the patient no distress, and, more important still, that, as it is given with the principal meal, it is subjected to the activities of the stomach when that organ is stimulated to do its utmost. For, as Moritz observes, the pill, being relatively heavy, will remain in the stomach the maximum length of time and so fully test the activity of the gastric juice. The desmoid will, therefore, often give a positive result, while with the test breakfast we get an absence of free hydrochloric acid. This is, as Sahli pointed out, a matter of considerable diagnostic importance in distinguishing cases with true achylia, as in carcinoma or pernicious anæmia, from less serious disorders in which the stimulus of the Ewald breakfast is insufficient to cause an excess of hydrochloric acid.

It is a test for both free hydrochloric acid and pepsin, and a positive reaction means that these two constituents are present in sufficient amount to digest the meal with which the capsule was given.

On the other hand this test is not suited to the distinguishing of various functional gastric disorders, and it gives us little clew as to any loss of motility. It does not, therefore, take the place of the stomach-tube and test breakfast, but acts as a control in those cases where the analysis of the test breakfast shows an absence of free hydrochloric acid or of enzymes, and so separates the mild from the severe cases of gastric insufficiency, fulfilling the function Sahli originally claimed for the test.

The most important result obtained up to this time with the test is that, of all the cases of cancer of the stomach and of pernicious anæmia which have thus far been examined in this way, and in which the test breakfast showed an absence of free HCl, in practically none did the capsule open.

Various other methods of *testing the gastric juice without using a tube* have been proposed. (The reader is referred to Herschell, "Manual of Intra-gastric Technique," 1903.)

**Pepsin.**—The qualitative determination of pepsin is less important than is that of hydrochloric acid, since pepsin is practically always present when there is any, and usually when there is no, free hydrochloric acid; that is, the pepsin-forming function of the stomach seems more resistant than the hydrochloric acid function, and the water secreting function most resistant of all. Its determination is,

therefore, limited to those cases without the free acid—carcinoma, pernicious anæmia and atrophic gastric catarrh. Schiff found that in benign simple hypacidity and anacidity the amount of pepsin does not change much, but that it is considerably diminished in cancer, even when the acid is only slightly reduced.

**Qualitative Determination.**—The presence of pepsin is assumed if the acid gastric juice (HCl added if none be free) will digest egg albumin or fibrin. The fibrin is prepared as follows: Fresh ox-blood is whipped and the fibrin kept in running water until perfectly colorless. It is then cut in fragments of equal size, put for a few days in alcohol, and then for one or two days in cool concentrated neutral carmine solution until fully stained. It is then well washed and pressed and kept in glycerin stained with carmine. Before use the fibrin is well washed with water to remove all glycerin. For egg albumin, the egg is boiled until perfectly coagulated (about five minutes, not longer). The white of the egg is then cut into 5 mm. cylinders, using an ordinary cork borer, and these into disks 1 mm. thick. These disks are kept in glycerin, and should be washed in water before using.

To gastric juice is added hydrochloric acid, if necessary, until the Congo-red paper shows free acid, then a few fibrin or egg fragments, and the whole put in a thermostat. If pepsin is present the fibrin will show signs of digestion in from fifteen to thirty minutes, the egg albumin in one-half to four hours. If fibrin is used the liberation of the carmine is a very early sign of beginning digestion. The first sign for the egg albumin disks is the rounding of the edges. Riegel prefers egg albumin to fibrin, since one gets more constant results as regards time and it is easier to control the mass used. Sahli recommends that both be tried since some gastric juices can digest the fibrin easily, but not the albumin. He recommends the following:

Tube 1: 5 cc. gastric juice plus fibrin.

Tube 2: 4.5 cc. gastric juice, 0.5 cc. 2 per cent. HCl and fibrin.

Tube 3: 5 cc. gastric juice plus 0.05 gm. pepsin and the fibrin.

Tube 4: 4.5 cc. gastric juice plus 0.5 cc. 2 per cent. HCl, pepsin and the fibrin.

Tube 5: 5 cc. gastric juice plus 5 cc. water.

Tubes 6 to 10: The same series, but using egg albumin.

Sahli recommends that these tubes be left at room temperature, that the difference in them may be more clearly observed. If in tubes 1 and 6 digestion is present in a half to three-quarters of an hour pepsin is normal and the hydrochloric acid normal or increased. The same is true if tubes 2 and 3 are not better than 1. Only rarely does one get complete digestion without free acid and then it is probable that lactic acid acts in its place. But in this case tube 2 will show better digestion.



Very rarely tube 3 is the best; usually tube 4 is when any pathological condition exists. When dilution improves the digestion (and then 5 is best) it means that motility is deficient.

**Quantitative Determination.**—The general law of ferment action is that its activity (as shown by the products of its digestion) varies as the square root of the amount of the ferment. The truth of this formula has been often tested. Caudet determined the nitrogen of the coagulable and uncoagulable proteid by the Kjeldahl method. Schütz and Huppert as the result of very careful work consider the formula  $s = ka \sqrt{p.t.s.}$  to hold ( $k = \text{constant}$ ;  $s = \text{deuteroalbumose}$ ;  $a = \text{albumin used}$ ;  $p = \text{pepsin}$ ;  $t = \text{time}$ ;  $s = \text{amount of HCl}$ ).

Hammerschlag used a 1 per cent. albumin solution with 0.4 per cent. free HCl which he put in two tubes, 10 cc. in each. To the one he added 5 cc. of water, to the other 5 cc. of gastric juice. These were left in the thermostat one hour and the coagulable albumin determined by Esbach's tube. Normally, 80 to 95 per cent. of the albumin is digested.

**METT'S METHOD.**—This test has been used by many,<sup>6</sup> and the conclusions were that pepsin varied as much as HCl. But it was soon found that the digestive power was decreased in the presence of sodium chloride or carbohydrates, or the products of proteid digestion. (This inhibition of the ferment is best seen in cancer and chronic catarrhal gastritis.) Hence Nierenstein and Schiff advised to always dilute the fluid sixteen times, that pepsin in this dilution may obey the formula. Egg albumin is filtered and the gas removed by a suction pump for several hours. A beaker is filled with the albumin, a bundle of glass tubes, each about 10 cm. long and 1 to 2 mm. wide, are then filled with the albumin by immersing them in it. Beaker and all are then put for five minutes in water at 95° C. The tubes are then carefully removed, their outside cleaned, and both ends closed with sealing-wax. One cc. of the filtered gastric juice is mixed with 15 cc. of twentieth-normal HCl and well shaken. In this fluid the tubes, cut into 2 cm. pieces, remain for twenty-four hours in the thermostat. The square of the average length multiplied by 16 will give the units of pepsin. (By unit is meant the amount of pure pepsin which in twenty-four hours will digest an average of 1 mm. of the albumin in the tubes.) In these dilutions they found the length of the column digested varied from 0 to 4 mm. The length which theoretically pure pepsin would give is 4 mm., yet the undiluted gastric juice often gave from 4 to 6 and even 8.6 mm.

Volhard digests an HCl-casein solution by the gastric juice. The casein is then precipitated by  $\text{Na}_2\text{SO}_4$  and the filtrate titrated. The total acidity will be higher the more the casein is in uncoagulated form

<sup>6</sup> E.g., Roth, *Zeitschr. f. klin. Med.*, Bd. 39, p. 1.

and the increase in acidity will vary as the square root of the pepsin. By this method the least trace of pepsin can be determined.

GROSSE'S METHOD.—A somewhat similar method was recently proposed by Grosse (*Berl. kl. Wochschr.*, 1908, vol. 45, p. 643). This test is based on the fact that pure casein is precipitated from solution by acetic acid, but the pus ducts of its digestion are not.

One gramme of casein (*Caseinum purissimum*, Grubler, prepared by Hammarsten's method) and 16 cc. of 25 per cent. hydrochloric acid (specific gravity 1.124) are dissolved in one litre of water on the water bath.

Ten cubic centimetres of this casein-HCl solution are measured into each of a series of test tubes. Graded amounts of the gastric juice are added to these tubes, the amounts depending on the juice. (Evidently the author dilutes and then adds amounts equal to 0.02, 0.03, 0.04, etc., of the juice.)

These tubes are then placed in the thermostat at 40° C. for just 15 minutes (or left in a water bath heated to 40° C.) and then several drops of a concentrated solution of sodium acetate added to each. The presence of still undigested casein will be demonstrated by a clouding of the fluid. Of the tubes which remain perfectly clear that containing the least amount of gastric juice contains just enough pepsin to digest all the casein.

A unit amount of pepsin is the amount contained in a gastric juice, exactly one cubic centimetre of which is sufficient to digest all the casein in 10 cc. of the 0.1 per cent. solution in just 15 minutes. If 0.025 cc. of the fluid is sufficient it would be said to contain 40 units; if 0.2 cc. is sufficient, 5 units, etc.

Grosse found that the average number of units for the gastric juice of apparently normal persons varied from 30 to 50.

He finds that the Schütz-Borissow law,  $Q = \sqrt{t} p$ . ( $Q$  = amount of casein digested;  $t$  = time;  $p$  = amount of pepsin) not to be true; but that  $Q = t p$ . That is, double the amount of pepsin and you double its digestive action.

ROSE'S MODIFICATION OF THE JACOBY-SOLMS METHOD.\*—In these methods one adds diluted gastric juice in varying amounts to a set of test-tubes, all containing the same volume of a turbid globulin suspension. The number of units of ferment is reckoned on the basis of that tube in which the least amount of gastric juice clears the fluid of all turbidity.

Jacoby and Solms† used a suspension of ricin; Rose, a globulin obtained from the ordinary garden pea, *Pisum sativum*, which is prepared as follows: Finely ground peas, freed as much as possible from the outer coating, are repeatedly extracted with large quantities of 10 per cent. solution of sodium chloride. The extracts are combined, strained through fine bolting-cloth, and allowed to stand overnight in large cylinders to deposit insoluble matter. The supernatant fluid is siphoned off and saturated with ammonium sulphate. The precipitate of albumins and globulins is filtered off, suspended in a little water, and dialyzed in running water for three days, to remove the salt and dissolve the albumins. The globulins are filtered off and washed two or three times with water to remove the last trace of albumins. To purify further, the precipitate is extracted with 10 per cent. solution of sodium chloride and filtered until perfectly clear. The

\* Arch. of Int. Med., 1910, vol. v, p. 459.

† Zeits. f. klin. Med., 1907, vol. lxxiv, p. 159.

resulting solution is exactly neutralized to litmus paper by the cautious addition of dilute sodium hydroxide, and again dialyzed in running water for three days to remove the salts completely. The precipitated globulins are then filtered off, and dried on a water-bath at 40° C. During the whole process of separation the proteins should be preserved with a mixture of alcoholic thymol and toluene. The globulins so prepared dissolve almost completely in a 10 per cent. solution of sodium chloride, and after slight acidification with hydrochloric acid yield a turbid solution which does not clear on standing.

The complete method as modified is as follows: 0.25 gm. of the globulin, prepared as described above, is dissolved in 100 cc. of a 10 per cent. solution of sodium chloride (by warming slightly if necessary) and filtered. (Such a solution will keep perfectly for two months if covered with a thin layer of toluene.) One cc. of the clear filtrate is introduced into each of a series of eleven small test-tubes about 1 cm. in diameter. To each tube is added 1 c.c. of 0.6 per cent. hydrochloric acid, and about five minutes are allowed for the development of the turbidity. A measured volume of the stomach contents is then exactly neutralized to litmus paper with dilute alkali. If a precipitate of acid protein forms, this is filtered off, and the clear neutral solution is diluted a known number of times (usually five) with distilled water, allowance being made for the dilution of neutralization. A portion of the diluted gastric juice is boiled and filtered. To each of the tubes are now added the boiled gastric juice, and then rapidly the unboiled gastric juice in the amounts indicated in the following table:

Tubes.....	1	2	3	4	5	6	7	8	9	10	11
0.25 per cent. globulin solution, cc.1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
0.6 per cent. HCl, cc.....	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Boiled gastric juice, cc.....	1.0	0.9	0.8	0.7	0.6	0.5	0.4	0.3	0.2	0.1	0.
Unboiled gastric juice, cc.....	0.	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9	1.

In clinical work tubes 3, 5, 7, 8, and 10 may be omitted. Each of these tubes contains exactly 3 cc. of the mixture and of each mixture the acidity is 0.2 per cent.

The solutions may be easily and accurately measured with a pipette of 1 cc. capacity graduated to 0.01 cc. The tubes are well shaken and allowed to stand in a thermostat or water-bath for fifteen minutes at a temperature of 50° to 52° C., or at a temperature of 35° or 36° C. for one hour. At the end of the digestion time that tube in the series is selected which exhibits no turbidity and which contained the least gastric juice. The peptic activity is calculated on the basis of the amount of gastric juice used in this tube. The enzyme content is expressed by the number of cubic centimetres of the 0.25 per cent. globulin solution that would be digested by 1 cubic centimetre of the undiluted gastric juice under examination, if the activity

should be exerted one hour at 35° or 36° C. or for fifteen minutes at 50° or 52° C. For example, if 0.5 cc. of a gastric juice diluted five times clears up one cubic centimetre of the 0.25 per cent. globulin solution in fifteen minutes at the given temperature, the activity of the solution would be expressed:

$$\text{Peptic activity} = (1 \div 0.5) \times 5 = 10.$$

The "normal" peptic activity on the scale here proposed is about 10, corresponding with the figure 100 on the scale of Jacoby and Solms.

In the case of the above method the conditions are constant, in all the test, in respect to acidity, volume, protein content, and temperature.

**The Fat-Splitting Ferment.**—*Volhard's Method.*—The yolk of one egg is mixed with 30 to 40 cc. of water; 10 cc. of this mixture are mixed with the gastric juice, both fluids being warmed separately in the thermostat, and then the mixture placed in a thermostat at from 37° to 40° C., then cooled. Seventy-five cc. of ether and a few cubic centimetres of alcohol are then added and the whole shaken out. A measured amount of this fat-containing ether is mixed with 50 cc. of neutral alcohol and titrated with tenth-normal NaOH, phenolphthalein as indicator. The result is the fatty acid. To the titrated mixture are now added 10 cc. of tenth-normal NaOH and placed on a water-bath for two hours. The flask is connected with a condenser and a calcium oxide tube to exclude CO<sub>2</sub>, or is allowed to stand for twenty-four hours in a closed flask at room temperature. This is to saponify the unsplit fat. Ten cc. of tenth-normal HCl are now added to free the fatty acid and the mixture again titrated with tenth-normal NaOH, phenolphthalein as indicator, to determine the fat which had been unchanged in the thermostat. From the relation of these the percentage of split fat is reckoned, and hence the number of units of ferment present. This ferment is very sensitive to alkali. It has been shown that its activity varies as the square root of the amount of the enzyme. Stade's formula is:  $p = \sqrt{f} \times \sqrt{t}$  in which  $p$  equals the products of digestion,  $f$  the units of ferment, and  $t$  the time. If  $f$  represents the amount of ferment which will split 1 per cent. of the fat in one hour, then, if after three hours one finds six per cent. split, twelve units of ferment must have been present.

Volhard found that in two hours from 30 to 36 per cent. of the neutral fat in the stomach was split. The fatty acids liberated, dissolved in the bile, evidently aid in the emulsion of the neutral fats, so that the stomach may do considerable of the work usually attributed to the pancreas. Certainly the demonstration of lipase in the gastric juice throws considerable doubt on the value of test meals to which neutral fat is added on the supposition that fat remains unchanged in the stomach.

It has been found that in hypochylia and achylia the lipase either is diminished in amount or is absent.

Riegel believes that much of the attention which has been directed to the acidity of the gastric juice should in the future be directed to the ferments of the stomach.

RENNIN.—The presence of rennin in the gastric juice is proved by the ability of this secretion, after it has been neutralized, to coagulate milk without change of reaction.

Leo's method of determining the presence of rennin is to mix from 3 to 5 drops of the gastric juice with from 5 to 10 cc. of fresh milk. [Riegel advises that from 5 to 10 cc. of gastric juice (neutralized with N/10 NaOH) be mixed with from 5 to 10 cc. of fresh milk.] In either case the tube or beaker containing the mixture is placed in a thermostat. Coagulation should take place in from 10 to 15 minutes. If a longer time is required, one should exclude coagulation brought about by lactic acid which has been formed.

The variations in the amount of rennin seem to run parallel to those of pepsin. Since the former is the easier ferment to estimate it may be that in time we shall follow its secretion rather than that of pepsin.

A quantitative determination of the amount of rennin present may be made by mixing in a series of tubes equal volumes of fresh milk with the same volumes of various dilutions of the neutralized gastric juice, and noting the highest dilution of the juice which will coagulate the milk. Boss found this to be in normal cases a dilution of 1 : 100 to 150. In cases of hypochylia the ability of the rennin to coagulate the milk may disappear when the juice is diluted 1 : 5 to 10. The only practical use for the quantitative determination of rennin is that suggested by Glassner, who found that in some cases of pyloric cancer the rennin secretion is normal and that of pepsin diminished, while in cancers of the fundus both rennin and pepsin are diminished in amount.

THE PRODUCTS OF PROTEIN DIGESTION.—We may divide the products of the peptic digestion of proteins into the following groups: albumoses, peptones and the products of further cleavage. The soluble albumin is precipitated by heat, the albumoses by the addition of an equal volume of saturated zinc sulphate and the peptones by phosphotungstic acid. The filtrate after this last precipitation will contain all products below the peptone stage of digestion. These various fractions of digestion-products are best determined by estimating the nitrogen in each filtrate, care being taken to reduce all quantities into terms of the original volume of the stomach contents obtained.

Benedict<sup>8</sup> advises to determine the precipitates volumetrically by centrifugalizing them to their smallest possible volumes. The meal used should contain but one proteid and this should be carefully weighed. We have used nutrose (a casein preparation) with good

<sup>8</sup> Am. Jour. Med. Sci., 1904, vol. cxxvii.

success. The nitrogen fractions of the contents of the fasting stomach of the patient should be determined in order that the necessary correction may be made. The meals should be given at the same hour of the various days, and removed at the end of the same period of time.

From a theoretical point of view it is interesting to note that in cases of carcinoma of the stomach digestion of proteid is so much more rapid than normally that it is safe to conclude that an abnormal ferment is present.<sup>9</sup> This is rendered very probable, since artificial digestion experiments with the heated and unheated carcinoma tissue show a similar relation.

In our benign cases tested in this way, the Ewald breakfast being used, the average amount of nitrogen in albumose form was 51.7 per cent.; in the phosphotungstic acid precipitate, 31.4 per cent.; in the residue, 16.9 per cent. In the carcinoma cases these figures were respectively 27.5, 47, and 27.6 per cent.

**Starch Digestion.**—It has recently been again emphasized<sup>10</sup> that the saliva is not as unimportant in starch digestion as was formerly supposed, and that it renders soluble in the stomach from 50 to 70 per cent. of the starch, thus relegating the starch digesting function of the pancreas to a subordinate position. This digestion occurs, however, before total hydrochloric acid has reached 0.12 per cent., hence is inhibited in cases of hypersecretion and hyperacidity. Concentrated lactic acid also can inhibit this process.

The stages of starch digestion are soluble starch, erythrodextrin, achroodextrin, and maltose. The relative amount of this may be approximately detected by the use of a very weak Lugol solution, since the later products of starch digestion have a greater affinity for the iodine than have the earlier. The colors obtained vary from blue to colorless, the first blue-violet, the erythrodextrin red to mahogany-brown. One drop of the weak Lugol's added to a small amount of gastric juice will therefore give no blue color with starch if much achroodextrin be present. On the other hand, if very few of the higher products be present, it will give a distinct blue color. From the number of drops to be added before the blue color appears may therefore be approximately determined the relative extent of the starch digestion. This has been considered a good indication of the amount of free acid, since in cases with high acidity the first drop may give the end reaction. And yet it should be remembered that the saliva is also to be taken into consideration; that of smokers, for instance, is supposed to have less digestive power than normal.

**Lactic Acid.**—The test for lactic acid is of value only when it is known that the meal contained none, and that if any is formed from any constituent of the meal it would normally have disappeared at the time of the test. Riegel says its presence in the stomach is never normal except during sugar digestion. One usually tests the juice

<sup>9</sup> Deutsch. Arch. f. klin. Med., vol. lxxii. p. 415.

<sup>10</sup> J. Müller, Verh. d. XIX. Congr. f. inn. Med.

after an Ewald breakfast, but this is hardly fair (see page 379). Dock uses a shredded wheat biscuit.

Some breads contain lactic acid, hence Boas proposes a meal of one tablespoonful of oatmeal cooked in one litre of water with a little salt. The stomach is well washed out and this meal given in the evening and removed the following morning. It is said that normal saliva can produce lactic acid from this meal. This meal is little used.

Uffelmann's test is the one in common use. This solution is always made up fresh. To about 20 cc. of 1 per cent. carbolic acid in a test-tube is added one drop of 10 per cent.  $\text{Fe}_2\text{Cl}_6$ . A deep amethyst color is produced. This is diluted with distilled water until the fluid is fairly transparent. It is then halved. To the one test-tube is added a drop or so of the gastric juice, to another the same amount of distilled water. If lactic acid be present, in the former the fluid takes a definite yellow or yellowish-green (canary-green) color.

Others propose 10 cc. of a 4 per cent. carbolic acid in 20 cc. of distilled water and one drop of the ferric chloride; others one drop of the ferric chloride solution diluted with distilled water until almost colorless, and then the carbolic acid (2 to 4 per cent.) added until the proper color is produced.

It should be noted that decolorization alone is not sufficient. A definite canary color or yellow is necessary. The blue is merely for contrast, hence one may dispense with the carbolic acid. It is well to control the test with dilute lactic acid.

The test as given by Strauss is particularly valuable. In a small separating funnel (see Fig. 63) with two marks, one indicating 5 and the other 25 cc., are introduced 5 cc. of the gastric juice. The funnel is then filled to the 25 cc. mark with alcohol-free ether and well shaken to extract the lactic acid. One drop of hydrochloric acid may be added to liberate that lactic acid which is bound to proteid. The gastric juice is then allowed to run

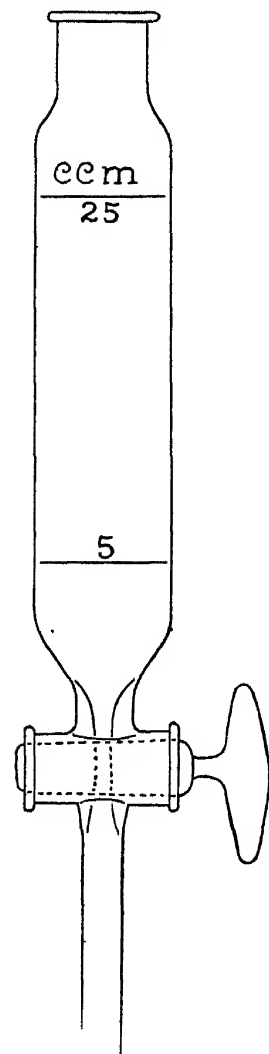


FIG. 63.—Strauss' separating funnel for lactic acid tests.

out and 5 cc. of distilled water added, then 2 drops (from a medicine dropper to insure uniform size) of a 1 per cent.  $\text{Fe}_2\text{Cl}_6$  solution. If 0.1 per cent. of lactic acid be present, the water layer will take a definite canary-green color. This test is perhaps not as delicate as some others; but it may be said confidently that if positive a pathological amount of lactic acid is present. Always to extract with ether is quite necessary,

since simple decolorization or even a suspicious color may be given by sugar, proteid, and alcohol, and by some other inorganic acids (oxalic, citric, tartaric), and positive tests may be prevented if much phosphates or peptones are present. Again, ferric chloride may give a cloud in the gastric juice which will obscure the test. If, however, the result of an Ewald breakfast given on a clean stomach be examined, trouble from these sources will not occur.

This test has been further modified. Kelling<sup>11</sup> dilutes the fluid with twenty volumes of water and then adds one or two drops of 5 per cent.  $\text{Fe}_2\text{Cl}_6$ . The tube is then sighted by transmitted light, and it is stated that from it 1 of lactic acid in 10,000 to 15,000 of water can be detected. Knapp<sup>12</sup> adds 1 cc. of gastric juice to 5 cc. of ether and shakes. He then superimposes this extract upon a colorless ferric chloride solution (1:2000) freshly prepared, and gets a canary-colored ring.

De Jong<sup>13</sup> adds to 5 cc. of the gastric juice one or two drops of  $\text{HCl}$ . He evaporates this to a syrup over a free flame and then extracts the residue with a little ether. The volume is then made up to 5 cc. with distilled water and one drop of 5 per cent.  $\text{Fe}_2\text{Cl}_6$  added, and the whole well shaken. A definite green color is produced by 0.05 per cent. lactic acid.

**Quantitative Lactic Acid.**—For clinical purposes the Strauss modification of the Uffelmann's method is accurate enough, and perhaps of greater value than the more accurate methods, which might show small amounts normally present. It is only the pathological amounts that are of value. The Hehner-Maly method is satisfactory if one consents to consider all organic acids as lactic.

Some idea of the amount may be obtained by comparing the acidity of the gastric juice before and after extracting it with ether.

**Boas Method.**—The principle of this method is to oxidize lactic acid to aldehyde and formic acid. The aldehyde is then changed to iodoform and this determined.

Either 10 or 20 cc. of the filtered gastric juice in a porcelain dish are evaporated to a syrup on the water-bath. If free  $\text{HCl}$  be present,  $\text{BaCO}_3$  is added in excess to prevent its volatilization. To the syrup a few drops of phosphoric acid are then added to again set the acid free and then the syrup heated to drive off the  $\text{CO}_2$ . It is then cooled and extracted two or three times with 50 cc. of ether. After standing about half an hour the ether is poured off, evaporated to a residue, this taken up with 45 cc. of water in a flask, then shaken and filtered. To the filtrate are added 5 cc. of concentrated  $\text{H}_2\text{SO}_4$  (sp. gr. 1.84) and a knife-point of manganese. This flask is then fitted to a distilling apparatus with two tubes through its cork, the one for the cooler and the other for the clamped rubber tube through which air may later be blown in order to cleanse the cooling tube from the aldehyde, and the fluid distilled until about four-fifths has passed over. The other end of the cooling tube dips into a well-closed Erlenmeyer flask containing 10 to 20 cc. of tenth-normal iodine solution in 20 cc. of  $\text{KOH}$ . This is shaken, closed tightly, and allowed to stand for a few minutes. The aldehyde will be changed to iodoform. To it are then added 20 cc. of  $\text{HCl}$  (sp. gr. 1.018) and an excess of sodium bicarbonate. It is then titrated with a tenth-normal sodium arsenite or tenth-normal thiosulphate solution, which has been accurately

<sup>11</sup> Zeitschr. f. physiol. Chemie, 1903.

<sup>12</sup> New York Med. Jour., August, 1901.

<sup>13</sup> Arch. f. Verdauungskranheiten, Bd. 2.



standardized to the tenth-normal iodine solution, until decolorized. A little fresh starch solution is then added, and one titrates back to the first permanent blue. The amount of iodine solution less that of the tenth-normal thiosulphate solution gives the amount of the former necessary to form the iodoform. One cubic centimetre of the iodine solution equals 0.003388 gm. of lactic acid. Or the iodoform may be estimated gravimetrically (see page 199).

**Other Organic Acids.**—Acetic, butyric, and valeric acids occur and are recognized by their odor. They are the result of bacterial decomposition, although the reason why in some cases one, in some another, is formed is not known. For instance, in a recent case the patient after an Ewald test meal complained of the taste of vinegar and the gastric contents were found rich in acetic acid. This man had given all the symptoms of hyperacidity.

Much organic acid could come from the foods, from fermentative processes in the stomach, or from the action of the fat-splitting ferment.

On a lactic acid-free meal, and only such should be used, one should normally find no lactic acid in the stomach. Lactic-acid-producing bacteria are in the mouth, but should not have enough time to produce the acid in the stomach.

Acetic acid carefully neutralized with soda is tested with 1 or 2 drops of  $\text{Fe}_2\text{Cl}_6$  solution, which gives the bluish-red color of ferric acetate.

Butyric acid in the presence of fine fragments of  $\text{CaCl}_2$  separates in fine oily droplets.

**Total Organic Acid. *Hehner-Maly Method.***—The principle of this method, which has been recommended by many, is that if a mixture of organic and inorganic acids be ashed the inorganic salts will remain neutral, while the organic will be changed to alkaline-reacting carbonates. The original acidity minus the final alkalinity may be considered to equal the mineral acidity.

Ten cc. of filtered gastric juice are neutralized with tenth-normal  $\text{NaOH}$ , using phenolphthalein as an indicator (number necessary =  $a$ ). This fluid is then evaporated and ashed, the ash taken up with water and titrated with tenth-normal  $\text{HCl}$ , phenolphthalein again used as indicator (amount necessary =  $b$ )  $a - b$  = the mineral acidity.

The method is easy. By it the acid phosphates are included with  $\text{HCl}$ , hence in its original purpose as a method of determining  $\text{HCl}$  the phosphates must be separately determined; but it is the easiest method to determine the organic acids.

**Bases of the Gastric Juice.**—Of these sodium is the most important. V. Mehring has shown that the stomach can and does secrete sodium carbonate to control the amount of hydrochloric acid. Reissner showed an increased secretion of alkali in cancer.

A base present in no small amount is ammonia (normally 0.1 to

0.15 p. m.). Of the tissues of the body which have been examined, the gastric wall contains the most ammonia. The fact that there is so much ammonia in the gastric contents throws considerable doubt on the accuracy of all the titrations in which phenolphthalein is the indicator used.

**FERMENTATION.**—Two kinds of fermentation take place in the stomach—that with gas formation, and that with lactic acid, but no gas, formation. Neither occurs unless considerable stasis is present. That with gas formation is found in cases with free HCl in the gastric juice, but rarely when this free acid is much diminished in amount or absent. In such cases lactic acid fermentation is more common.

The occurrence of lactic acid fermentation is proved by the presence of this acid in the gastric contents, provided foods can be excluded as its source. Small amounts of it may be found when there is no genuine stasis, as after a heavy meal, or if there are in the stomach wall pockets or little clefts. In testing for gas fermentation two fermentation tubes should be filled with the gastric contents, or with vomitus, well shaken and unfiltered. To the contents of one of these tubes, which serves as a control, as all the carbohydrate of the material tested may already have been fermented, is added a little glucose. The tubes are then left in the thermostat for twenty-four hours (some say for three or four days), at the end of which the amount of gas, if there is any, is noted. Both carbohydrates and proteids may ferment in the stomach. The test has some value in determining the degree of stagnation, that is, the abundance of gastric flora. The organisms of fermentation which are found in the stomach are yeasts, sarcinæ, and long bacilli. Sarcinæ are present in cases of high acidity, bacilli in cases of low acidity and of anacidity. Some believe that sarcinæ with a low acidity, or a low acidity and long bacilli, indicate cancer of the stomach. Yeasts are common. Coyon doubts whether sarcinæ explain much of the fermentation, and he has isolated two bacilli, the enterococcus and *Coccus radiaire*, which he thinks are important in this connection.

Among the products of fermentation have been found formic, lactic, and acetic acids, the higher fatty acids, ethyl alcohol, aldehyde, ammonia, hydrogen disulphide, and carbon dioxide.

Hydrogen disulphide, from the decomposition of proteid, is rarely found in the stomachs of patients with malignant, but frequently in the stomachs of patients with benign, stenosis of the pylorus. Some foods, as radishes and onions, and also the saliva must be excluded as its source. This gas may be recognized by its odor, or by suspending over the fluid a strip of paper moistened with alkaline sugar of lead. Its presence is not indicative of the existence of any particular organism in the stomach.

MICROSCOPIC EXAMINATION.—The inspection of the partially digested food seldom yields any valuable result, for under normal conditions much of the starch and muscle enters the bowel unchanged. Some try to determine the degree of deficiency of gastric digestion by noting the number of muscle fibres whose cross striation is well preserved, but this is a criterion of doubtful worth. Mucus and pus can best be recognized through the microscope. Pus in the stomach in amounts sufficient to make it recognizable on macroscopic examination is a very rare find. It has been found in quantities varying from 60 to 500 cc. in cases of gastric cancer and of subphrenic abscess perforating into the stomach, but these cases are rare (Strauss). One would expect to find it in gastritis phlegmonosa and in local abscess of the wall of the stomach.

A few pus cells can be found microscopically in the washing of practically every empty normal stomach. (Only in the washings of a fasting stomach which previously has been cleaned by lavage of all particles of food can these cells be recognized.) Large numbers of leucocytes can be found on microscopic examination in cases of gastric cancer. The diagnostic value of this finding is doubtful.

Fragments of gastric mucosa are found in the stomach contents removed with a stomach tube, provided strong suction is exerted through the tube by a suction pump. On the other hand, those whose technic in emptying the stomach through a tube is uniformly careful find the presence of these bits of mucous membrane a valuable hint as to that vulnerability of the gastric mucosa which is present in malignant disease, in chronic gastritis, and in some cases of Heynoch's purpura with œdema of the wall of the stomach (Morris). The fragments of proliferating mucosa thus obtained in some cases of achylia gastrica closely resemble bits of carcinomatous tissue.

Fragments of tumor should be searched for in the morning stomach washing of patients suspected to have gastric cancer. Among the other interesting finds in gastric washings are groups of leucocytes whose nuclei form characteristically-shaped groups, and whose protoplasm has been digested; snail-like, spiral-shaped masses of myelin altered by HCl; crystals of cholesterolin, of leucin, of calcium oxalate, and, in case bile has flowed back into the stomach, of triple phosphate; parasites' eggs, yeasts, sarcinæ, and bacilli.

On the whole, one gains little information by the microscopic examination of the gastric contents. The fragments of tumors, chains of long bacilli, and parasites' eggs are the most valuable finds.

INFUSORIA (see page 424) are sometimes found in the gastric contents of patients whose gastric juice is steadily neutral or alkaline in reaction.

The greatest variety of BACILLI are of course found. EPITHELIAL CELLS occur often in abundance, particularly when digestion is poor. CANCER FRAGMENTS are very rare. If free hydrochloric acid be present, MOULDS and YEASTS and SARCINÆ will predominate; if none, BACTERIA. Einhorn found in the wash-water spores of moulds which he thought lodged in crevices of the mucosa and might aid in the hyperacidity and the gastralgia. Yeasts are abundant in gastric dilatation, and a few may be found in normal stomachs. Sarcinæ occur in large numbers in benign dilatation, occasionally in gastritis, ulcer, and neuroses, but rarely in cancer. One observer reports them in such numbers as to form plugs obstructing the pylorus. Ehret found many sarcinæ in cases of intense fermentation, but neither yeasts nor bacteria, and considers that they indicate a severe stasis. He does not consider that they occur only with free hydrochloric acid. Two sizes may be found, the large and the small. In a recent case in this clinic with dilated stomach the sarcinæ were in great numbers, and of huge size.

BLOOD.—The œsophagus, nose, mouth, and lung as a source must be excluded. Small amounts of blood have no significance, since slight lesions of the pharynx, œsophagus, or stomach are very easily produced in vomiting or by the tube. Blood may be present in large amounts in ulcer of the stomach or in rupture of venous dilatations at the cardiac orifice as in liver disease. In case the stomach be empty, the blood will then be arterial, but as a rule it is dark because of the hæmatin produced by the gastric juice, and is clotted and well mixed with food. In carcinoma of the stomach there is usually a slight constant oozing from the tumor. This blood is well mixed with the food, digested, and looks like coffee-grounds. This is a very valuable sign in cases of gastric dilatations and indicates cancer of the mucosa. It also occurs in hyperacidity with hemorrhagic erosions of the mucosa. The detection of this small amount of blood is no easy matter, since microscopically nothing is found. Blood may arise from tuberculous ulcers; from slight injuries to the mucosa; from small aneurisms of the gastric arteries; and from the digestion of the mucosa over an infarcted area. Gastric hemorrhage occurs in chronic passive congestion; cirrhosis of the liver; various constitutional diseases without apparent reason, as in anæmias, hæmophilia, Hodgkin's disease; in active hyperæmia as in vicarious menstruation; and following abdominal operations, especially such as involve the omentum, in which case they are a disturbing symptom, but of no moment. All the blood of even a profuse hemorrhage may pass by the stools.

Occult hemorrhage discovered by examining the gastric contents and stools occurs in several conditions. Boas and Kochmann<sup>16</sup> divide cases as follows: Those with no hemorrhage; gastritis anacida, sub-

<sup>16</sup> Arch. f. Verdauungsk., 1902, vol. viii. Heft 1, 2.

acidity, hypersecretion, benign ectasis; cases with blood at times, especially ulcer; cases with blood as a rule, especially cancer.

**Deen's Test.**—To the gastric juice is added 1 cc. of fresh tincture of guaiac and 1 cc. of Hühnerfeld's solution (glacial acetic acid, 2; distilled water, 1; oil of turpentine and alcohol, of each, 100 cc.). The mixture is well shaken. If blood is present the fluid turns blue. The test is also given if iron compounds are present, or some vegetables, etc., hence it has chiefly a negative value. Weber's modification is recommended by Riegel: to the gastric contents is added one-third volume of glacial acetic acid and it then shaken out with ether. After the ether extract has cleared, to a few cubic centimetres are added 10 drops of guaiac tincture and 20 to 30 drops of turpentine. Blood will give a bluish-violet color. In this case only raw or rare-cooked meat is to be excluded.

Heller's test is also used, but various red substances must be excluded, coffee, cocoa, wines, etc. The precipitate should be collected on a filter and dissolved in acetic acid. Rhubarb, senna, and santolin may deceive.

For the spectroscopic test the gastric contents are diluted with water, a few drops of concentrated acetic acid added, and then shaken out with one-fifth volume of ether. In a few minutes a clear layer of brown ether solution of hæmatin is obtained. The four-band spectrum of hæmatin in acetic acid could be due to chlorophyll, hence alcoholic KOH is added and it reduced with  $(\text{NH}_4)_2\text{S}$ , the red fluid will now give the two-line spectrum of reduced hæmatin.

**Absorption Power of the Stomach.**—Although this function of the stomach may not be important, yet there are certain substances which are absorbed, as alcohol, sugar, dextrin, peptone, albumoses, *et al.*, and in amounts varying with the quantity in the stomach. The absorption test generally applied is that of Penzoldt. On an empty stomach is given a gelatin capsule containing 0.2 gm. of potassium iodide. The saliva and urine are examined every few minutes until the test is positive. To the saliva or the urine are added a little starch meal and fuming  $\text{HCl}$ . The blue color will indicate the presence of iodine. Or a little starch meal is added and crude  $\text{HNO}_3$ , but an excess of acid must be avoided or the first trace of iodine will be lost. Also the sputum and starch must not be left in contact long as the digestion will give erythrodextrin and achroodextrin. Chloroform may be used instead of starch, and will take up the iodine to a pink solution. Normally the excretion begins in six and one-half to fifteen minutes in the sputum, and in the urine in thirteen and one-half minutes. The time is shortest on an empty stomach and at the height of digestion. The test should be used under constant conditions, hence Sahli combines it with the Ewald breakfast, in which case

iodine appears in five to twenty-five minutes. The test has a theoretical, but very little practical value. Only a considerable delay counts. There is a delay in most gastric diseases. This may occur in dilatation with considerable catarrh, and carcinoma; there is no delay in ulcer without catarrh, nor in neurotic disturbances. Potassium iodide is not food, and it is not absorbed from a stomach which is closed off from the intestine, hence the test may show when it first reaches the intestine.

Again, of importance is the SECRETION OF WATER, which is the most resistant function of the gastric mucosa and may be stimulated independently of the other secretory functions. It is increased owing to the presence and absorption of alcohol, sugars, etc. This "dilution secretion" is supposed to be a measure to protect the intestine, the increased fluid diluting the gastric contents.

**Motility of the Stomach.**—It is to be emphasized that disturbance of the motility of the stomach is far more important than disturbance of its secretions. If motility be good the intestine can vicariously make up easily for any insufficiency on the part of gastric secretion, and the person live years in ignorance of the fact that he has no gastric juice. But if motility be impaired the stagnation of food in the dilated stomach soon and always produces serious results.

Hypermotility is seen especially in cases of hyperacidity, and it may be necessary to remove the Ewald breakfast in from one-half to three-quarters of an hour in order to obtain any fluid. But the most rapid motility is seen in cases of jejunal fistula high up. In these cases of starvation the stomach seems to try to hurry the food into the intestine at as rapid a rate as possible. In one such case a glass of milk was drunk and collected at the fistula. It appeared in one minute, and was wholly recovered in four minutes from the time it was swallowed. The surgeons used the following method of determining the position of the fistula. An oyster was tied with a piece of silk thread and swallowed. In a few minutes it appeared at the abdominal opening. The thread was then cut at the teeth, pulled through and measured.

It is quite important to wash the stomach out to be sure it is empty, since in cases of achylia the tube may siphon nothing, but the wash-water may show considerable solid matter.

Megalogastria means enlargement of the stomach, and may or not be accompanied by motor insufficiency.

Ectasis refers to enlargement with motor insufficiency; it is "atony" if due to real weakness of the muscle wall, is "hypertonic ectasis" when due to pyloric stenosis.

Motor insufficiency may be absolute or relative and dilatation of the stomach is in general due to one of two factors,—(1) atony of the gastric wall, in which case the muscle is not strong enough to

empty the stomach; in this group are found the largest stomachs. Strauss reports a case<sup>17</sup> of five and a half litres capacity. (2) Muscular insufficiency, that is, an obstruction at the pylorus which renders exit of the food difficult. In such a case the muscle wall may be abnormally strong and hypertrophied; in others there is no dilatation of the stomach, since the wall is extensively hypertrophied.

Stenosis at the pylorus causing dilatation may be congenital or acquired. Acquired stenosis may be due to the contraction of scars of ulcers; to cancers; to hypertrophic stenosis which is said to be the result of continued pyloric spasm stimulated by hyperacid gastric juice or an ulcer; to the scars resulting from irritating poisons; to the pressure of neighboring tumors; to twists; to diverticula; and, finally, to malpositions of the pylorus resulting from adhesions.

In a normal case, no matter how large the meal, the stomach should be empty in seven hours. A common test of motility is to give without previous lavage a simple evening meal, but one of constant composition, as of cold meat, bread and butter, and tea (Boas). If the following morning food be found, there is considerable motor insufficiency. If before this evening meal the stomach had been well washed out and found empty in the morning, the degree of insufficiency is less; if food is found, even more. If the stomach contains food seven hours after a full noon or morning meal, but none after a night's rest, the degree of insufficiency is least.

Ewald and Strauss have recommended to give one spoonful of currant or raisin preserve with the evening meal. The seeds of this can be recognized in the stomach washings the next morning, no matter if the patient has taken a large breakfast.

If in the morning the fasting stomach contain over 100 cc. of fluid, motor insufficiency may be suspected, but such a stomach will be empty in the morning if the evening before it had been washed clean.

The symptoms of dilatation are those of the disease causing it, and the vomiting of large amounts of food which has been eaten more than seven hours, in some cases even three days, before. The vomiting of food in the morning before breakfast is a sure sign.

Another test for those patients who object strenuously to a stomach-tube is that of Ewald and Sievers. It is based on the belief that salol remains unchanged in the stomach, but is split by the pancreatic juice and bacteria to salicylic acid and phenol. The salicylic acid is excreted in the urine as salicyluric acid, which may be easily detected by the violet color on the addition of ferric chloride to the urine. The test assumes that the time of splitting, absorption, and excretion remains constant, which is not always true. One gramme of salol is given with the test breakfast and the urine is examined at intervals. The test should appear in the urine at the latest in seventy-five minutes.

<sup>17</sup> Deutsch. med. Wochenschr., 1904, No. 15.

If the first appearance is delayed longer there is certainly motor insufficiency. But one cannot be sure that the salol will mix with the food in the stomach. It may enter the intestine with the first portions of food or with the last. Again, in cases of stagnation of the stomach contents, bacteria can split some of the salol, and mucus will also, so Huber recommends to test not the time of the appearance but of the disappearance of the salicyluric acid. Its output should cease in from twenty-six to twenty-seven hours. The urine is therefore examined first twenty-seven hours after the meal, and if found, at intervals of three hours. Sahli recommends to determine the time both of its appearance and disappearance. While this is a very gross test, it does give a certain amount of information. In cases with disturbed motility, especially in those with simple atony and pyloric stenosis, the test may not appear for several hours, and may continue for even forty hours.

Jodipin has also been recommended by Winternitz.<sup>18</sup> Pancreatic secretion and the bile are necessary to split iodine from this fatty compound.

To determine the amount of residue in the stomach various methods have been proposed, such as the introduction of 100 gms. of olive oil (Klemperer) and the removal of as much as possible in two hours, washing well with water and separating the oil in a separating funnel. This method is severely criticised since the oil does not mix uniformly with the contents

Sörensen and Brandenburg<sup>19</sup> recommend to give on the empty stomach 300 to 500 cc. of 3 per cent. ptyogen. Of this there is removed as much as possible in from one-half to one hour. From 100 to 200 cc of water are then introduced and again removed. The nitrogen in both fractions is determined by the Kjeldahl method, and from this the contents calculated.

The composition of the gastric juice in dilated stomachs will depend on the disease causing the dilatation. In general there are two groups of cases,—one with acid and the other with anacid contents. The former includes cases of ulcer and of continuous secretion, the latter those of cancer and chronic gastritis. As a rule the mucosa of a dilated stomach becomes less sensitive to stimuli, owing perhaps to the constant presence of food and to the gradual development of a chronic gastritis, and so secretes less acid.

Of 45 of our benign casts, 7 were hyperacid, 15 showed normal acidity, 9 hyp acidity with, and 4 without, any free hydrochloric acid.

By "hyperacidity" is meant the secretion of abnormally acid gastric juice during digestion, that is, while there is normal stimulus for secretion. By "hypersecretion" is meant a secretion of gastric juice in amount out of proportion to the physiological stimulus, or when this is absent. Hyperacidity is qualitative, hypersecretion is quantitative, yet they usually coexist. Hyperacidity often exists without symptoms, is, in fact, often a constitutional anomaly; hypersecretion is always pathological and produces pathological results (Riegel).

**Hyperacidity, Supercidity, Hyperchlorhydria, or Hyperaciditas Hydrochlorica.**—This secretion of very acid juice during digestion, but not on the empty stomach, involves not alone the total acidity, but especially the increased free hydrochloric acid. There might, indeed,

<sup>18</sup> Zeitsch. f. physiol. Chemie, vol xxiv.

<sup>19</sup> Arch. f. Verdauungskrankheiten, Bd. 2.



be a high total acidity due to a large amount of organic acids which would not come under this head.

This may be due to nervous causes, to defective nervous control, or to changes in the mucosa. After the Riegel meal the motility is found normal or even increased (hyperkinesis), and the stomach empty at the end of six or seven hours, and sometimes even in three to four hours, the food being discharged into the intestine before it is ready. The acidity per cent. is often 100, sometimes 150 to 160 or more following the meal, and over 70, and even 100 or more, with the test breakfast. The free acid after the meal is 60 to 80, after the breakfast from 50 to 60. Organic acids are absent. Others (Meunier) say the acidity alone is not important, but the specific gravity must be low, 1007 to 1019 instead of 1022 to 1040, as normally.

The digestion of meat is excellent, but not that of starch. Absorption is good. Some of these cases are certainly functional, while others are secretory neuroses. For this latter diagnosis all causes for gastric disease must be excluded, and the increased acidity should vary with the nervous symptoms and be very variable, hence the term "heterochylia" (Hemmeter), while in chronic gastric disease the acidity is quite constant. The hyperacidity may be present only during certain periods and following certain nervous stimuli, an important point in a diagnosis at best difficult.

**Hypersecretion, Supersecretion, Continuous Secretion "Gastro-succorrhœa."**—In continuous secretion the secretion continues when the stomach contains no food. If the bread of the Ewald breakfast be given without the water, and much is removed, it means true hypersecretion due to a disproportion between stimulus and response—the free acidity is relatively high, which is a valuable point in ruling out motor insufficiency. Continuous secretion is determined by finding much acid gastric juice in the fasting stomach and without anything to indicate stasis. To exclude food the stomach must be well washed. This condition may be constant or intermittent, a part of a general neurosis, a secretory neurosis, or the result of organic nervous disease. Among the last may be mentioned the gastric crises of tabes dorsalis; among the first are cases of gastroxynsis (Rossbach).<sup>20</sup> It is seen in neurasthenia and hysteria, myelitis, general paralysis, and even the excessive use of tobacco. In the periodic or intermittent cases (Reichmann's disease), during the intervals the person's digestion may be perfectly normal. Then occur sudden pains, acid eructations, and the vomiting of a cloudy yellowish fluid, first with food, then pure, often several hundred cubic centimetres of fluid of normal or increased acidity, the latter usually only when food is present (total acidity 30 to 50, abundant free HCl).

<sup>20</sup> Deutsch. Arch. f. klin. Med., Bd. 35.

Cases of continuous secretion usually have a gradual onset and a long course. The chief symptoms are a feeling of discomfort and of weight in the epigastrium, eructations of acid fluid, a pain which begins about an hour after eating and increases until the stomach has emptied itself into the duodenum, or the patient has vomited. Vomiting about midnight is a very characteristic symptom. The patient usually vomits large amounts (from 500 cc. to 1000 cc. or much more) of a thin fluid the acidity of which is normal or slightly above the normal. There is often pain before meals, which is relieved by eating.

Many doubt whether continuous hypersecretion is ever "functional." They claim that it is always caused by some gross stimulus to secretion, as ulcer, stenosis of the pylorus, or by some condition favoring the retention of particles of food.<sup>21</sup>

Foster and Lambert\* have shown that pyloric stenosis is a sufficient cause of continuous secretion; that the presence of retained food particles is not necessary to explain it.

To diagnosticate chronic hypersecretion, we must repeatedly find 100 c.c. or more of clear fluid in the stomach an hour or more after we have carefully cleaned it by lavage and emptied it as completely as possible. (Some consider 50 cc. the upper limit of normal; in some cases 1000 cc. are found.) Riegel recommends the following routine. The stomach is emptied at the height of digestion after a full meal, and its contents are measured. The next step is to pass the tube some morning after a night during which the patient has eaten and drunk nothing and the quantity of clear fluid obtained is measured. Lastly, the stomach is washed and emptied (very carefully, since it is hard to wash out all the food) some evening, and the contents obtained the following morning are noted.

After the test meal one often gets over one litre of contents, with a total acidity per cent. from 90 to 100, free HCl 50 or more (sp. gr. 1004 to 1006.5). A case reported by Thayer is a good illustration. It was of two years' duration; the total acidity after the Ewald breakfast was 113; the fasting stomach always contained even 420 cc. of acid fluid, acidity per cent. often 117; digestion was good.

Riegel considers that atony alone is not sufficient to explain hypersecretion. Surely the reverse is quite as often true. Hyperacidity and hypersecretion cause spasm of the pylorus, and as a consequence, failure of the stomach to empty itself, and hence dilatation. In several cases of dilatation of the stomach relieved by operation the condition soon recurred, showing that the secretory abnormality was the basis of the trouble (Riegel). It must also be admitted that such

<sup>21</sup> Kaufmann, *Am. Jour. Med. Sci.*, 1904, vol. cxxvii.

\* *Jour. of Exp. Med.*, 1908, vol. x, p. 820

stomachs are abnormally sensitive to stimulus, while in simple dilatation with stasis the mucosa does not respond with normal irritability, but seems dulled by the constant presence of food.

Other such cases ordinarily called nervous are supposed to be reflex disturbances from the intestine and are relieved by treating this organ.<sup>22</sup>

**Nervous Dyspepsia.**—Hyperacidity, hypersecretion, anacidity, are conditions which may accompany a variety of disturbances, and as terms they refer only to the chemical composition of the gastric juice. These abnormalities of secretion may be due to organic changes of the mucosa, to functional disturbances following bad habits of eating, poor food, etc., or be a part of a general neurosis, "nervous dyspepsia." In this country the last is an exceedingly common manifestation of neurasthenia which stomach specialists abroad speak of as the "American disease." Yet it is exceedingly difficult to separate the element due to food, rapid eating, etc., from the neurotic element, and in the majority of cases perhaps both coexist. There is usually good reason for gastric distress, and a neurasthenic will often worry his subliminal gastric sensations into the sphere of consciousness.

Some of these "neurasthenics," if one tests their gastric juice, have hyperacidity; more, slight subacidity; and many, normal conditions. It is interesting that their subjective symptoms bear so little relation to the condition of the gastric juice. A patient with hyperacidity may describe sensations quite similar to those of an anacid case, unless there is vomiting in the former case, which feature the latter is, as a rule, spared; and one with apparently normal gastric juice sometimes complains as much as either of the others.

In this clinic during the past four years we have had 300 such cases. We have made no effort to separate "functional" cases from the purely neurotic. Eighty-two were cases of hyperacidity (this includes the cases of supersecretion and continuous secretion; all figures quoted are those of the Ewald breakfast). Of 20 others the clinical features were hyperacidity, although the total acidity was not over 70. In 36 cases the total acidity was 70 to 80, the free 33 to 69 (the majority from 45 to 55); in 21, from 80 to 90, free acid 32 to 68 (the majority 55 to 65); in 15, from 90 to 100, free acid 53 to 85; in 10, from 100 to 110, free acid 60 to 89. As regards amount of fluid obtained one hour after the test breakfast, over 100 cc. were obtained in the first group (total acidity 70 to 80) in 30 per cent. of the cases; in the second group, in 35 per cent.; in the third, in 20 per cent.; while in the group with total acidity over 100, in 29 per cent.

Subacidity (total acidity less than 40) was present in 170 cases, in 61 of whom there was no free hydrochloric acid. In these 61 cases the total acidity was seldom over 20, in 18 was 10 or less, and in 4 the fluid was practically neutral to litmus. It is of interest that in these subacid cases in but 4 per cent. was more than 100 cc. obtained, while in 8 per cent. nothing could be siphoned off at the end of one hour. In 148 cases the gastric juice was found practically normal. This may illustrate the lack of parallelism so often seen between the sensations and the chemical findings, but one must also consider the possibility that

<sup>22</sup> Faber, Arch. f. Verdankhtn, Bd. 7.

the test breakfasts were not given at the best time to observe the abnormality in secretion.

**Acute Gastritis.**—By this is meant an acute irritation or inflammation of the superficial layers of the mucosa, resulting in increased mucus secretion, or desquamation of the epithelium, and disturbance of secretion. It may be primarily due to the direct irritation of foods, poisons, or intoxications, heat, cold, etc., or, secondary to various chronic diseases. The vomitus of these cases is acid in reaction, of a bad odor, often fermented, the food undigested as a rule and with much mucus. The total acidity is diminished, free hydrochloric acid absent as a rule, and often organic acid present. Rarely is the reaction neutral. If there has been much retching, the vomitus is bile-stained or even pure bile. A test meal will show mucus, undigested food, and little or no free HCl.

We have records of but five good cases, all with subacid or neutral fluid.

**GASTRITIS PHLEGMONOSA OR INTERSTITIAL PURULENT GASTRITIS** is a very rare condition. It is an inflammation of the entire gastric wall even to the serosa. When localized it gives rise to gastric abscess. Vomiting is present, as a rule. In the 60 diffuse cases reported, however, pus has not been present (Riegel), but has been in a very few cases of abscess.

In the **GASTRITIS ACUTA PURULENTIA** (Leube) the inflammation is limited to the mucosa.

**Chronic Gastritis.**—Chronic gastritis is not nearly as common as is its diagnosis. It exists in all grades to atrophy of the mucosa. Functional disturbance must first be excluded, and only those cases included in which there are definite signs of gastritis with increased mucus formation. One of the commonest symptoms is vomiting, especially on an empty stomach in the morning or at the height of digestion, of undigested or poorly digested food mixed with mucus. If on the fasting stomach, it consists of bile-stained mucus, well seen in the morning vomiting of alcoholics.

The test meal must be tried. The amount removed is about normal. The food has the appearance as if just swallowed, and much mucus is present which is intimately mixed with the food. This renders its removal through the tube difficult and its filtration tedious. The presence of this mucus is indispensable for the diagnosis, since large amounts from the stomach indicate catarrh. The macroscopic appearance is the best standard for amount. To judge the amount of mucus, however, the stomach must be thoroughly washed, since the most appears in the later washings. Hence it is that the vomitus is so often

deceptive in this particular. The needle douche tubes are often valuable.

The secretion is usually diminished, and in late cases with atrophy of the mucosa there may be no secretion at all. Free hydrochloric acid is diminished or absent, but the total acidity varies and for short times the free acid may return, hence the necessity of repeated examinations. There are cases of acid gastritis, but they are rarely diagnosed, though this may be due to the fact that they are an early stage before the patient consults a physician.

Of our 27 cases, one was slightly hyperacid (72, total acidity); in 10 the acidity was within normal limits, and in 15 below 40, nine of whom had no free acid. Four of these could be termed atrophic catarrh, and one additional case, from which stomach could be obtained by the tube but 1 cc. or more of bile-stained mucus, at autopsy was found to be a case of cirrhosis of the stomach.

For the diagnosis of a gastritis acida it is necessary to find in the fasting stomach mucus with many cell nuclei and a hyperacid juice.

In chronic gastritis the secretion of gastric juice is diminished. The diminution progresses as the case advances, until there may be complete achylia. The secretion of hydrochloric acid is affected first; that of the ferments second (Bouveret believes that the easiest way to follow the progress of a case is to observe the rennin secretion); that of water next; while all this time the secretion of mucus even increases. Proteid digestion is much impaired, carbohydrate digestion not at all. The stomach often becomes somewhat, seldom much, dilated, since its walls are weakened, and the pylorus is sometimes slightly obstructed by inflammatory thickening of its mucosa. The presence of much undigested food in the stomach washings does not necessarily indicate much, if any, stasis, as it is a function of the stomach to retain food until it is digested. If gastric motility is impaired fermentative processes will arise, but seldom more than a trace of organic acid is found in the contents. In many cases the gastric motility is normal or even increased, and then there are very few symptoms of stomach trouble, as the intestine vicariously fulfills the gastric functions. In cases of chronic gastritis the mucosa of the stomach is abnormally fragile, and one often finds fragments of mucous membrane in the stomach washings.

**Mucus.**—A little mucus is usually demonstrable in the contents of normal stomachs, especially towards the end of digestive periods. Mucus is present in excess under the following circumstances: if the diet is particularly rich in starch; in conditions of subacidity and anacidity of the gastric juice, though in these cases the increase is sometimes only apparent, for, while the normal amount may be secreted, an amount less than normal may be digested; if the stomach contents are irritating to the mucosa, which seems to protect itself

in this way; rarely in cases of gastric hyperacidity, and these may belong in the preceding group; in all forms of chronic gastritis ("gastric catarrh"), both the primary form and that which accompanies cancer of the stomach, pyloric stenosis, etc. The largest amounts of mucus are found in cases of developing achylia. It would appear that the secretion of mucus increases as that of the gastric juice decreases. A correct idea of the amount of mucus present in the stomach can be gained only by repeatedly filling and emptying the stomach, or, better still, by the use of a needle douche-tube, for the mucus sticks tenaciously to the gastric wall.

Mucus from the stomach appears as delicate transparent flakes, which are mixed with the food, which sink in water, and which contain the nuclei of leucocytes. Mucus of the air passages appears glassy-looking, in balls, which float (they enclose air), which often contain visible pigment and cylindrical epithelium microscopically visible. They are not mixed with the food. Foster\* has shown the importance of mucus in explaining the diminution of, and variations in, the amount of free HCl in cases with a high free acidity, for the products of the digestion of mucus have a high acid-binding power.

**Atrophy of the Mucosa. Achylia Gastrica.**—Achylia gastrica may be due to a functional disturbance of an apparently normal mucosa or to real atrophy of the mucosa; and the latter, the end stage of a chronic gastritis or the result of cancer and other diseases which lead to degenerative changes in the mucosa. When due to atrophy it is a gradual process, the secretion diminishing until finally there is almost no gastric juice. The diminished secretion may be due to a very small cancer, and much of the mucosa seem intact; it seems due to some toxic substance from the tumor. Achylia gastrica can exist when there is cancer in other organs (breast, intestine, œsophagus, uterus, *et al.*), and before any disturbance of the general health. In conditions with general malnutrition achylia may be present. The cases of particular interest are those which resemble pernicious anæmia. Their gastric trouble may not be suspected provided the motility of the stomach be good, yet if slight atony also exist the gastric symptoms will be evident enough. If the motility is good the intestine will act vicariously.

Some cases are of nervous nature. Einhorn reports such a case of five years' duration of achylia and then a return to normal. The discovery may be purely accidental. On the other hand, the nervous symptoms may disappear, leaving the achylia still in evidence.

Atrophic stomachs are very susceptible to injury, and it is not uncommon in the washings to get pieces of mucosa which seem to show a granular gastritis. Such cases vomit often, not always, and,

\* Am. J. of Med. Sci., Feb., 1907, vol. cxxxiii; and Med. Record, Aug. 13, 1910.

as a rule, soon after eating, the vomitus consisting of undigested food, and is almost never bloody. For diagnosis of achylia the test meal is necessary, and this perhaps is the condition in which the test meal gives the most positive results. It should be repeated several times. It is impossible to remove much. The food is little changed, the total acidity is very slight, from 1 to 4. There is no free HCl. Lactic acid is rare unless there be severe ectasis. To diagnose the anatomical condition is more difficult. The ferments may fail, an important point in diagnosis. It is easy in washing out the stomach to get fragments of mucosa and traces of blood showing the vulnerability of the mucosa. Mucus is, as a rule, absent, yet early there may be much, and later the mucosa may consist chiefly of mucous cells, the glandular cells having disappeared. To obtain nothing through the tube does not necessarily mean an empty clean stomach, since by washing the dry contents may be removed. Elsner,<sup>23</sup> *et al.*, consider the vicarious action of the intestine to be overrated; undigested food should be retained, and if the stomach is washed out this will be found.

Elsner's method of measuring motility is to wash the stomach out well one hour after the test meal. This fluid is allowed to stand in a graduated cylinder for twenty-four hours, then the amount of residue read after several decantings. If there is over 210 cc. of residue there is motor insufficiency in addition to the achylia.

**Ulcer of the Stomach.**—The clinical types of this disease are:

(1) The latent form which may pass unsuspected or until hemorrhage or perforation occurs.

(2) The hemorrhagic form, which may be acute and sometimes fatal, or chronic, causing considerable anæmia and cachexia resulting from the frequent small hemorrhages, the stools always containing a certain amount of blood.

(3) The acute perforative.

(4) The chronic dyspepsia, in which case the dyspeptic symptoms are the most evident, and the characteristic symptoms of ulcer vary, the most important chemical signs being the hyperacidity and the absence of mucus.

(5) Neurotic, or gastralgic type.

(6) The vomitive form, with vomiting as the worst symptom.

(7) The cachectic form, which presents the picture of a cancer.

The cardinal symptoms of this disease are: (1) Increasing dyspepsia, usually of long duration. (2) Pain, paroxysmal and local, from half an hour to two hours after the meal when peristalsis is most actively rubbing the food across the ulcer. (3) Vomiting of well-digested food and acid vomitus, usually one to three hours after the meal at the height of the paroxysm, but often in the morning also

<sup>23</sup> Deutsch. med. Wochenschr., 1904, No. 42.

since the juice is hyperacid, and is followed at once by a diminution of the pain. (4) Blood is only at times present, and in from 30 to 50 per cent. of the cases. As a rule, it is dark in color, due to the hæmatin formed by the hydrochloric acid, although if the stomach be empty it may be arterial. There is often blood in the stools intimately mixed with the food, but not as constantly as in cancer. In case the blood rests a long time in the stomach the vomitus may be of coffee-ground appearance, but in that case iron, wine, coffee, medicines and food must be excluded. In many cases the blood of the stools is unsuspected. Other sources of hemorrhage must be excluded,—tuberculosis, cancer, chronic passive congestion, cirrhosis of the liver, rupture of an œsophageal varix. (5) Hyperacidity is a classical symptom and yet Ewald says it is present in but about half the cases. The digestion is good and motility usually rapid. In an average of 75 cases tested with the Riegel meal he found the total acidity to average 105, the average free HCl 50, with a maximum of 89. But the acid may be diminished because of other diseases of the mucosa, catarrh, etc. These two groups must be distinguished, the fresh and the old ulcers, for in the latter the acidity is much lower.

The result in such cases may be stricture, or severe anæmia due to the insufficient nutrition, vomiting, or hemorrhage. In case the blood is lost chiefly by the stools the ulcer may be unsuspected and the case be diagnosed as pernicious anæmia. Cancers may develop on the bed of the ulcer,—in fact, some think the majority of cancers begin thus,—and at the time of death the acid still be considerable. In most cases it gradually diminishes until the picture is typical. One examination of the gastric juice is never enough. Repeated examinations must be made in order to get a general idea of the acidity. In the case of developing cancer it is the variable yet diminishing acidity which is important, and yet cases of ulcer may have a normal or diminished amount of hydrochloric acid.

The 82 cases of this clinic have been reported by Howard.<sup>24</sup> Vomiting was present in 85.3 per cent., especially in the cases with ulcer at the pylorus. Vomiting of blood occurred in 75.6 per cent.; in one-third only was the blood bright red. After the test breakfast more than 50 cc. was obtained from 54 per cent., 27.5 per cent. showed hyperacidity, 42.5 per cent. subacidity (for these figures the acidity per cent. of 60 was considered the upper limit of normal). In 18 per cent. there was no free hydrochloric acid, in 14 per cent. lactic acid.

**DUODENAL ULCER** is often impossible to diagnose. Yet the position of the pain, its late occurrence after the meal, and the fact that all the blood will appear in the stools is suggestive. Hyperacidity may be present. These ulcers are more often latent than are the gastric.

**HEMORRHAGIC EROSIONS.**—It is doubtful whether there is for this

<sup>24</sup> Am. Jour. Med. Sci., December, 1904.



a characteristic complex which as yet can be recognized. The most valuable point is that in washing the empty stomach there have been found usually fragments of the mucosa without marked pathological changes. Vomiting is rare. The acidity is normal or diminished, rarely increased.

**Cancer of the Stomach.**—Clinically these cases may be separated into the latent, those with cachexia but no gastric symptoms, and those with localizing symptoms. The important diagnostic points of this disease are its rather sudden onset with dyspeptic symptoms in a person beyond middle life, unless it be a case that develops on the base of an ulcer the symptoms of which have long preceded it; loss of weight and strength; anæmia; pain; vomiting; and on chemical analysis lack of free hydrochloric acid, the presence of lactic acid and of the Oppler-Boas bacillus.

Of these local symptoms and signs, however, any one may be absent. Vomiting is common (in 85.3 per cent. of our first 150 cases), yet it depends upon the position of the cancer; if at the pylorus causing stenosis, the vomiting will be late after a meal; if at the cardiac orifice, there will be regurgitation at once after eating. There is least vomiting when the cancer is on the stomach wall. If there is no stenosis at either orifice, there will often be none, yet in 6 of 30 cases with the cancer at an orifice there was none. In those cases with dilated stomach due to stenosis the amount of vomiting is often from a half to one litre or more of food, in which may be recognized that eaten days before, in one of our cases four weeks (Osler and McCrae). The albuminous part of the food is poorly digested, hence the meat in lumps, with mucus often, sometimes decomposed and often with digested blood. Some hemorrhage is almost constant. It is parenchymatous as a rule, and yet it may be rapid and fatal. It is often an early feature, and leads to the diagnosis of ulcer. Often the patient will not know of the hemorrhage unless his stomach be washed out or his stools be carefully examined, since it is gradual and the blood by digestion takes on the so-called "coffee-grounds" appearance and is mixed with food. Of the 150 cases reported by Osler and McCrae, vomiting of blood occurred but in 21.8 per cent., but careful examination of the stools showed it present in almost 100 per cent.

The gastric features of cancer may be grouped as follows: First, those due to the pyloric stenosis, with subsequent dilatation of the stomach and stasis of the contents, hence fermentation, decomposition, etc. In some other cases the motility is excellent or even increased, as in 11 of 76 cases, in which it was hard to get any fluid at the end of an hour. Second, those due to the chronic degenerative changes of the mucosa which begin early and develop late; the gradual diminution in the amount of secretion, *e.g.* And lastly, those due

to the cancer itself. Among these are the early absence of free hydrochloric acid, perhaps the presence of lactic acid, and possibly the presence of the long bacilli in chains.

To consider first the symptoms due to the cancer *per se*; absence of free hydrochloric acid is an early and important sign of cancer, present in over 80 per cent. of cases at first examination, yet alone not of great importance, not even when other signs are present; in cases of pernicious anæmia, for instance, there are many features of cancer, and in gall-stones with the pylorus in a mass of adhesions, and hence palpable tumor, the free HCl may fail. In 163 cases of this clinic the free acid was absent in 146, or 89 per cent.

Again there may be a group of cases without previous symptoms of ulcer which begin with hyperacidity (Ziegler). The total acidity may not be in the least diminished and total chlorides be high. The acidity varies considerably from day to day, and, in fact, even with the presence of free acid a variable acidity, with sometimes free hydrochloric acid and another day none, may lead to the diagnosis of carcinoma.

This was well seen in a recent case early in the disease, and was a point leading to operation. In fourteen days, of the three Ewald breakfasts given the acidities were total 121, 100, 37, and free HCl 11, 16, and 10 respectively.

The lack of free acid is at first certainly due to the binding of this acid by some body which itself does not react alkaline to litmus, yet which prevents the hydrochloric acid from giving the Günzberg or other tests for it in its free state. The idea originally suggested by v. de Velden, that there was a secretion of the cancer which bound the acid, is the idea now again advanced. Later, as a result of the changes in the mucosa, brought about perhaps by this secretion of the cancer, the total amount of acid will gradually diminish to a very small amount; but it is to be emphasized that the conditions causing its absence vary early and late. Early with normal total acidity absence of the free hydrochloric acid is one of the most important signs; later the amount secreted is diminished. In these early cases it is very interesting that the disappearance of free acid may be sudden, and that following the excision of the cancer the free hydrochloric acid has returned a day or two later; again, in cases of carcinoma of the duodenum or œsophagus the disappearance of free hydrochloric acid can be best explained as due to fluid from the tumor which flows into the stomach; the cancer may be small and very local, but its effect considerable. What these bodies are which bind the free hydrochloric acid has been a subject of considerable investigation. Certainly hydrochloric acid, if introduced into the stomach of a carcinoma case, is soon neutralized (Stahelin). They

could be products of the digestion of protein—albumoses, peptones, and hexone bases. Reissner explained the disappearance of free acid in the presence of an undiminished or even increased total acidity on the theory that free alkalies are secreted by the tumor tissue. But if this were the case the chlorides thus formed would be neutral, and the total acidity would be reduced. Work which we have done leads us to believe that the tumor furnishes the gastric contents with a proteolytic ferment the action of which produces an abundance of digestion products which can bind the acid and yet allow it thus combined to react as acid to litmus. Fischer\* has confirmed this work by isolating from the contents of carcinomatous stomachs tyrosin, leucin, arginin, and lysin.

Later in the progress of the malignant diseases there is a gradual reduction in the amount of total HCl secreted, until finally the gastric contents may react as alkaline to litmus. On the other hand, the secretion of hydrochloric acid may for a time increase as the result of regular lavage, proper dieting, and general building-up treatment. In cases of cancer developing on the base of an ulcer the presence of free HCl may be noted for a considerable period. In such cases there are often marked fluctuations in the quantity of acid. To-day there is much of it, a few days later none, and later still the free acid is again present. These variations are thought to have great importance in diagnosis.

Of 64 of our cases without free hydrochloric acid, the juice after the Ewald breakfast was almost or quite neutral in 8, below 10 (acidity per cent.) in 20, between 10 and 20 in 15, between 20 and 50 in 14, and between 60 and 103 in 7. The high acidities seemed to depend on the lactic (and butyric) acid present.

Later the pepsin is diminished and the rennin as well. This is due to the chronic gastritis resulting in a diminished secretion of gastric juice, and is not specific for the cancer.

Lactic acid is often an early and very valuable sign in cancer. It occurs in about 90 per cent. of the cases sooner or later, when there is no free hydrochloric acid although the bound acid may be abundant. It is true that cases of cancer without lactic acid occur, *e.g.*, those on the base of an ulcer; also more rarely lactic acid without cancer, as in case of an atonic and anacid stomach, atrophic catarrh with stenosis of the pylorus and hence long stagnation of the gastric contents; but in the benign cases it is so often absent, even though the stenosis be extreme and the fluid anacid, that its early appearance in cancer, even before the stenosis is considerable and the total hydrochloric acid much diminished, means that these two factors cannot alone explain its appearance, and emphasizes the value given it by Boas in the early

\* Deut. Arch. f. klin. Med., April 24, 1908, Bd. 93, p. 98.

diagnosis of malignant disease, although the specificity he claimed is not granted.

In 609 of our cases without gastric cancer, lactic acid was present in 30. All were cases of subacidity with no free hydrochloric acid. These cases were: atrophy of mucosa, 1; chronic gastritis, dilated stomach, 4; ulcer, 6; nervous dyspepsia, anacidity, 3; pernicious anæmia, 2; gall-stones, 1; cirrhosis of liver with jaundice, 1; cancer of gall-bladder, 1; pulmonary and peritoneal tuberculosis, 3; and an interesting group of inflammations of the large intestine (ulcerative colitis, etc.), 5; cancer of ovary, 1; peripheral neuritis and fibrinous pericarditis, 1 each.

Riegel considers that for its appearance there must be, first a diminished secretion involving ferments as well as acid, then stagnation of the contents, and perhaps insufficient absorption. Yet he admits that the gastric juice of cases of cancer without much atony may contain considerable lactic acid, and that his experience of over twenty years shows that the presence of considerable lactic acid nearly always means gastric carcinoma. He explains one case without stasis by the fissures at the base of the cancer, which allowed the retention of acid-producing organisms. Hammerschlag considers that it appears only when the ferments are diminished, hence when proteid digestion is poor, and is a sign of the diminution in the secretion of gastric juice with stagnation and deficient absorption. This occurs particularly in carcinoma of the pylorus.

While the lactic acid may often be determined in a test breakfast, this is hardly a fair test since its formation cannot be as rapid as that of the secreted acids. It is best therefore to test the contents of the fasting stomach in the morning after it has been well washed out the evening before and a test meal given (see page 381).

The cause of the lactic acid may be the organisms in the stomach, since several of these have been proven to be acid-producing, among them the Boas bacillus; or it may be a normal product of digestion evident in these cases because of diminished absorption (an improbable explanation to cover many cases); or it may be the product of a specific ferment furnished by the tumor. This latter cannot be excluded, and in the autolytic digestion of proteid by ferments from these tumors lactic acid has been shown to arise.

In our cancer cases the fluid removed one hour after an Ewald breakfast was examined for lactic acid, hence the per cent. which it presents will be minimal. It was present in 63 per cent. of 137 cases. The figure given by Schiff was 73.5 per cent. of a group collected from various writers.

The gross appearance of the contents is of great importance, since the meat is poorly digested and the carbohydrates well. Disturbance of motility is due to mechanical obstruction at the pylorus. On the other hand motility may be excellent and yet digestion very poor.

which is true in early cancer not situated at the pylorus. Strauss claims in such cases we have an abnormal fermentation due to bacteria which have remained in the clefts of the tumors.

Tumor fragments are seldom found. Fragments in blood-clots washed from the stomach should be examined. Sahli emphasizes the possibility of diagnosis from washing out the stomach well at night and the fasting stomach again the next morning; in the latter wash-water the fragments of the tumor may be found. Considerable blood in various stages of digestion is common. In achylia gastrica fragments of mucosa may easily be washed loose and resemble cancer. In this clinic fragments were found in several cases, but the number depended on the care with which they are searched for, for in over 70 cases they were noted but twice, and in a few months after the attention of the clerks was called to the need of searching for them several cases were found.

Sarcinæ and yeasts are rare. In but five cases of our clinic were sarcinæ found. The bacteria of the stomach, which are probably a large



FIG. 64.—*Sarcina ventriculi* and yeast cells.  $\times 900$ .

group, have been divided into the "short" and the "long." The former occur in catarrh and ectasis due to benign conditions. The presence of many sarcinæ is evidence against cancer. That which has attracted the most attention is the so-called Oppler-Boas bacillus, which occurs in about 80 per cent. of cases (Rutinmeyer), and in almost no other condition than here. Cultural characteristics of this organism have not as yet been well worked out, and the cultures are so seldom made that as the Oppler-Boas bacillus we usually have in mind a group of organisms with a few points in common, especially the morphological characteristics; that is, a long, coarse, thread-like bacillus, often in long chains which extend across the field of the microscope, in some cases present in enormous numbers even filling the whole field. No spores are seen. The single bacilli are from 3 to 10 microns long (6 to 8 microns as a rule) and 1 micron broad, with rounded ends, often slightly bottle-shaped; some are bent. They stain by Gram's, and are best seen in stained specimens. Of course, few or many could be present, but we never speak of them as Oppler-Boas bacilli unless

they are abundant, coarse, and in chains. Some passing under this name are surely the Gas bacillus, but the Boas bacillus is not anaërobic, and has been found to grow best on media containing blood or its derivatives, and hence perhaps its presence so often and so exclusively in carcinoma, in which condition above all is present the coffee-ground vomitus rich in albumin detritus, the ulcerations and clefts of the mucosa, the failure of ferment formation, of acid secretion, and the stagnation, which factors Schmidt considers essential. These bacilli do not grow well on ordinary media, but will luxuriantly if blood be added. They coagulate milk. Kauffmann found the bacillus in 19 of 20 cancer cases, proved that it was a lactic acid builder, and found that it occurred in numbers proportional to the amount of lactic acid. Most other lactic acid bacilli are smaller. Other bacilli of similar appearance have been cultivated,<sup>26</sup> yet its diagnosis is chiefly morphological. Apropos of the number present, a recent case without extreme stasis may be mentioned, for the gross sediment of the stomach washings was almost entirely composed of masses of these bacilli. It is not fair to search for them in a recently washed stomach. If there certainly is stenosis and these are absent, the evidence is against cancer. Kaufmann<sup>27</sup> claims that they cannot grow in the presence of free hydrochloric acid of 0.02 per cent., but can well when the fluid is acid with phosphates and lactic acid; but others (Rosenheim) say they flourish in the stomach in spite of free hydrochloric acid.

Our series is hardly the right one to furnish statistics concerning the presence of these organisms, for only the fluid removed after an Ewald breakfast on a cleaned stomach was examined, yet these organisms were found in only 38 per cent. of 55 cases in which their presence or absence was noted. In four of these cases with the bacilli lactic acid seems not to have been present.

Heichelheim<sup>28</sup> thinks that in diagnosis clots of blood are very important. To find clots containing many of these bacilli, and a fluid without free hydrochloric acid, speaks very strongly for cancer; clots with few bacilli strongly suggest it; clots alone and repeatedly present speak in favor of it.

Pus is sometimes present; in fact, the largest amount of pus that we have seen in any gastric case was one of carcinoma. The resorption is much disturbed and the KI test almost always delayed.

Among other tests proposed for the early diagnosis of cancer of the stomach is the tryptophan test of Erdmann and Winternitz<sup>29</sup> which is not constant enough (Sigel in 2 of 15 cases; Glässner in 1 of

<sup>26</sup> See Schmidt, *Wien. klin. Wochenschr.*, January 10, 1901.

<sup>27</sup> *Centralbl. f. inn. Med.*, 1904, No. 4.

<sup>28</sup> *Zeitschr. f. klin. Med.*, 1904, vol. liii. p. 447.

<sup>29</sup> *Münch. med. Wochenschr.*, 1904, p. 299.

2) to be of great value and does occur in other conditions (ulcer, *e.g.*), yet it makes the diagnosis very probable (Orlowsky).

The presence of over 0.5 p. m. of albumin (Esbach), which Salomon considered the surest sign, and of urea in the washings of a fasting stomach without retention, are of some but not absolute value. The stomach is carefully washed out; then in a few hours the tube is again introduced and all possible removed, washing several times with 400 cc. of physiological salt solution. The albumin and nitrogen are then determined. In all other conditions  $N = 0$  to 16 mg. per 100 cc., but in cancer 10 to 70 mg. per 100 cc.: cancer is probable when  $N =$  more than 20 mg. and there is a definite albumin precipitate by Esbach's fluid. Common ulcer can be differentiated early, although it is the ulceration of the cancer nodule which furnishes this inflammatory exudate.<sup>30</sup>

Gluzinski's test for the relative insufficiency of HCl secretion by testing free hydrochloric acid in the morning on the fasting stomach, forty-five minutes after the test breakfast, and four hours after a full meal, is valuable also to indicate a cancer on the bed of an old ulcer, it being positive in 12 of 13 cases.

Infusoria, and especially flagellates, are sometimes present in the anacid carcinomatous stomach at a very early stage. Cohnheim<sup>31</sup> reported six cases with the *Trichomonas* and *Megastoma entericum*, and thinks this a valuable sign, even the first, for the early diagnosis of an ulcerating cancer of the cardia or lesser curvature; not in pyloric cancer, since the lactic acid would kill them. They are often present in our food and are a temporary inhabitant of the stomach until acid is excreted.

Zabel<sup>32</sup> reported four early cases with similar organisms present in abundance. Rosenfeld<sup>33</sup> found them in six cases, one of which he thinks is the first non-carcinomatous case in which they have been found. He expected this would be true of another case, but a cancer was later in evidence. They are found in the small amount of neutral or alkaline fluid of these fasting stomachs, together with *leptothrix* threads, long bacilli, and spirilla. It is interesting that they cannot be found in other cases of achylia, for we must often swallow them.

Blood in the gastric contents and stools is a very important, common (68 of 70 cases),<sup>34</sup> and early feature, especially in the absence of hydrochloric acid and when motility is good.

Strauss emphasized the disproportion between the relatively active fermentation and small amount of sediment in case the cancer is not at the pylorus; Reissner, the early increase of chlorides to almost or

<sup>30</sup> Berent and Gutmann, *Deutsch. med. Wochenschr.*, 1904, No. 28.

<sup>31</sup> *Deutsch. med. Wochenschr.*, 1903.

<sup>32</sup> *Wien. klin. Wochenschr.*, 1904.

<sup>33</sup> *Deut. med. Wochenschr.*, 1904.

<sup>34</sup> Boas and Kochmann, *Arch. f. Verdauungsk.*, 1902.

quite double, and the alkaline reaction of the ashed gastric contents. For Glässner's idea concerning ferments see page 371.

The early diagnosis of cancer of the stomach is unfortunately a late one, if one means by "early", that it is made at a time when the patient can be saved by operation. No one feature will help for even a fairly early diagnosis. The chemical features may be very suggestive, in some cases normal, in some even the reverse of those suggesting cancer. The surgeons insist that the diagnosis should be made before any tumor is palpable, and this should be the aim of the clinical chemist. At present we admit that age and clinical history are of far more importance than chemical examination. We would never delay operation until the clinical chemist found positive any test yet proposed, fearing that when it did become positive it would then be too late to operate.



## CHAPTER IV

### THE INTESTINAL CONTENTS AND FÆCES

To determine the **motility of the intestine** is often important, particularly in metabolism experiments to separate the stools belonging to the various periods; also in "latent constipation," one of the "new" diseases, in which the food is much too long in its passage through the intestine, but the condition overlooked since the stools are normal in number and size. It would indeed be fortunate did this condition remove the stigma of neurasthenia from some of our suffering patients. The normal motility after a mixed meal is from six to twenty hours; after milk, thirty-six to forty-eight hours. Charcoal or lycopodium powder is generally used, one drachm in water after a meal and the stools watched until the black charcoal is seen grossly, or the characteristic lycopodium spores microscopically. Or carmine, 0.5 gm., may be given and the red color watched for. Allowance should be made for the gastric motility in case the actual time in the intestine is desired. Sometimes the charcoal is so mixed in a considerable mass of fæces that it passes unnoticed, hence lycopodium or carmine is somewhat safer.

**Pancreatic Fluid.**—This when present in the duodenum may be obtained, according to Boas, by massaging the contents of the duodenum into the stomach previously washed with 1 per cent. soda solution. The patient lies on his back, and the abdomen is massaged from right to left from the costal margin to the parasternal line. The stomach-tube is then quickly introduced and whatever may have been forced back removed. Sometimes about 50 cc. are obtained. To prevent the destruction of the ferments by the hydrochloric acid, soda should be added at once. The presence of trypsin is assumed if fibrin or egg albumin is digested in alkaline medium.

**Trypsin.** ARTHUS AND HUBER'S METHOD.—Fresh fibrin is washed in water and then heated at 40° C. for twenty-four hours with 2 per cent. NaF. The solution of fibrin is then filtered. The intestinal fluid plus an equal amount of 2 per cent. NaF is mixed with two to three volumes of the above-mentioned fibrin solution and kept in the thermostat at 40° C. for some time. If trypsin is present the typical crystals of tyrosin will be easily found. Contamination with bacteria is not to be feared, for the fluid will remain sterile indefinitely.

The **FAT SPLITTING FERMENT** may be demonstrated as follows: Neutral olive oil is obtained by shaking out olive oil with ether and a little NaOH. The ether extract is shaken out repeatedly with water

and the ether then evaporated. In each of four test-tubes are mixed 10 c.c. of very dilute neutral litmus solution and 5 drops of an emulsion of the above-mentioned neutral oil (made by shaking together 10 parts of oil, 5 of gum, and 35 of water). The fluid to be tested is added, 2, 4, 8, and 16 drops, to each of the tubes, and these are left for a few minutes in a water-bath at 37° C. The presence of lipase will be shown by the red color in one or more of the tubes. The presence of *diastase* is proved if at the end of a few minutes after a little of the fluid has been mixed with a thin starch solution the addition of a drop of iodine solution fails to give a blue color.

*Test Meals.*—When studying intestinal conditions one should always use some test meal which he has already tried on healthy persons often enough to know what to expect under normal conditions. In work in metabolism this is very important. Folin's diet should be used by all who wish to get results comparable with his, and he is the only one who has published a complete analysis of the urine of patients on any standard diet.

The patient's daily diet is as follows: whole milk, 500 cc.; cream (18 to 22 per cent. fat), 300 cc.; eggs (white and yolk), 450 gm.; Horlick's malted milk, 200 gm.; sugar, 20 gm.; sodium chloride, 6 gm.; water, enough to bring the whole up to 2000 cc.; extra drinking water, 900 cc. This daily ration consists of about 119 gm. of protein, 148 gm. of fat, and 225 gm. of carbohydrate. It contains 18.9 gm. of N, 5.9 gm. of  $P_2O_5$ , 3.8 gm. of  $SO_3$ , and 6.2 gm. of Cl.

Others prefer a diet of milk alone or of milk and eggs for experiments in metabolism. In following a single patient through several periods of observations any diet will do, providing it is the same during all the periods; but if different patients are to be compared a standard diet is necessary.

McCrudden\* proposes a way of analyzing the food given in experiments in metabolism which is free from many of the objectionable features of the usual methods. Instead of the separate analysis of each of the foods consumed, they are all analyzed at once; food does not need to get stale while the experiment is in progress, for one can give a liberal diet of fresh food and change it when desired; the foods taken for analysis are all mixed together in the proportions in which they are given the patient, and one analysis of the mixture as a whole is sufficient.

In the case of liquid foods the fluids are mixed as thoroughly as possible, a certain volume is given the patient, and the same volume is taken for analysis. A solid food is mixed after being cut into

\* Jour. of Med. Research, 1903, vol. ix, p. 135.

small pieces. The patient receives a certain weight of it, and the same weight is reserved for analysis. By this method only non-homogeneous foods are excluded.

At the end of the experiment all the food reserved for analysis is well mixed together, and, after the addition of a little hypochloric acid to retain all the nitrogen, as much as possible of the water contained in the food is evaporated on the steam bath, and the remainder is removed by twice adding alcohol and continuing the evaporation. The food thus dried is next put through a grinder, and then, since it can more easily be reduced to a powder when free from fat, this is extracted with naphtha. It is now crushed fine, so that it will all pass through a fine sieve, and then its volume is reduced by quartering. That is, the food is thoroughly mixed with a large spatula and then made into a little circular pile two inches high. This pile is divided into four nearly equal quarters. Two opposite quarters are rejected, and the other two are well mixed together, made into another little pile, and quartered again. The mixing and quartering are repeated until there is about enough food left of one set of analyses.

For the macroscopic and microscopic study of the stools with a view to determining how well the various foodstuffs are utilized, the best diet is that of Schmidt and Strassburger.\*

Morning: 0.5 litre milk and 50 gm. zwieback. Forenoon: 0.5 litre oatmeal gruel strained (made from 40 gm. oatmeal, 10 gm. butter, 200 gm. milk, 300 gm. water, and 1 egg). Noon: 125 gm. chopped beef (raw weight) broiled rare with 20 gm. butter, 250 gm. potato broth (made of 190 gm. mashed potatoes, 100 gm. milk, 10 gm. butter). Afternoon: As morning. Evening: As forenoon.

This diet, which is given for a period of from three to four days, consists of 1.5 litres milk, 100 gm. zwieback, 2 eggs, 50 gm. butter, 125 gm. beef, 190 gm. potatoes, and 80 gm. oatmeal.

It contains about 102 gm. proteid, 111 gm. fat, 191 gm. carbohydrates. Its total caloric value is 2,234.

**The Digestive Power of the Pancreatic Juice.** —A method which has promised much is that of Sahli, who gives with an Ewald test-meal 0.15 gm. of iodoform in a glutoid capsule (gelatine hardened in formalin). This is supposed to be digested only by the pancreatic juice; iodine will appear in the sputum in from a quarter to one and a half hours after the solution of the capsule.

Unfortunately, Sahli does not give very explicit directions concerning the preparation of these capsules, and it would seem that they

\* *Die Fæces des Menschen*, Berlin, 1905. See also Hewes, *Boston Med. and Surg. Jour.*, April 8, 1909, vol. clx, p. 429.

could be obtained from but one source. This is unfortunate, yet it is for those interested in the success of the test a wise provision, since so much depends on uniformity in the preparation of the capsules.<sup>2</sup> For the pancreon test, see page 443.

In cases of *jejunal fistula* it is often of importance to know how near the pylorus is the opening. A convenient method is to tie a silk thread to an oyster. In these cases of practical starvation with the fistula high up, the motility of the stomach is excessive, as if that organ were trying to aid the body by sending the food at once to the intestine. In an interesting case in this hospital<sup>3</sup> the oyster appeared at the fistula's orifice, which, by measuring the length of the string, was found to be but one foot below the pylorus. In this same case, after drinking a glass of milk the milk began to escape from the fistula in one minute, and the total amount was recovered in four minutes.

**The examination of the stools** is much neglected. It is disagreeable but so valuable that it should never be overlooked. As the sputum examination is commonly limited to staining for the tubercle bacillus, so that of the fæces is now a matter of searching for parasites when they are suspected, with the result that much that is valuable passes undiscovered, and, when examined, the ordinary constituents, since not familiar, are misinterpreted.

For this examination are necessary a few tall glass jars in which the stools mixed with water are allowed to sediment, some strainers (colanders) of various sized mesh in which the stool is ground by a pestle, and plates half black half white, the same as used for sputum.

**The constituents of normal stools** are the undigested portion of food, bacteria, intestinal secretion, formed and unformed elements from the mucosa, salts, and products of digestion. The amount varies widely with the diet, but a general average is 120 to 250 gms. per day.

The relative amount which bacteria form is enormous. Strassburger<sup>4</sup> estimated them at about one-third the weight of dried stools, that is, eight grammes per day, and containing about one-half the nitrogen of the stools. He found more in some dyspepsias,—14 to 20 gms.; remarkably less in chronic constipation,—5.5 to 2.6 gms. Strassburger's method was as follows: 2 cc. of the stool is well mixed with water and centrifugalized; the organisms will remain suspended, the elements of the food sediment. The fluid is then poured off, considerable alcohol added to lower the specific gravity, and again centrifugalized. This time the bacteria will sediment. This sediment is then dried and weighed. Another 2 cc. are evaporated (fresh alcohol

<sup>2</sup> Sahli. Deutsch. Arch. f. klin. Med., 1898, Bd. 61.

<sup>3</sup> Cushing. Johns Hopkins Hosp. Bulletin, July, 1899.

<sup>4</sup> Zeitschr. f. klin. Med., 1903, Bd. 48, p. 413.

being repeatedly added), dried and weighed. Klein,<sup>5</sup> who uses a counting method but who does not even guess the relative volume of the bacteria in the stool, takes exception to Strassburger's method and results.

The small amount of fæces during starvation periods consists of bacteria, the intestinal epithelium, mucus, and the intestinal secretions.

**Reaction.**—The normal reaction is neutral, faintly acid, or faintly alkaline, especially the last. If urine be mixed with it, it is of course soon alkaline. In a mass of fæces the reaction at the surface may differ from that at the centre, and the changes on standing are very rapid. In typhoid or cholera they are alkaline, as a rule, in patients on a milk or starch diet they may be very acid.

**Frequency.**—By *diarrhœa* is meant frequent and fluid stools; by *constipation*, infrequent movements of the bowels, associated with symptoms which are relieved by purging. The normal stool is never fluid, but frequency is a variable matter and must be judged from the individual stand-point, hence subjective symptoms are necessary.

Diarrhœa may be due to increased peristalsis, increased intestinal secretion, or decreased absorption, and accompanies chronic enteritis, chronic peritonitis, intestinal tuberculosis, amyloid disease, cirrhosis of the liver, cholera, typhoid, dysentery, infectious diseases, uræmia, etc.

When the trouble is in the small intestine the movements are fluid and large but not necessarily very frequent; in dysentery they are frequent and scanty.

Constipation as a chronic condition is the result of careless habits of personal hygiene, of sedentary life, of a diet lacking in the constituents which stimulate intestinal peristalsis, of dilated stomach, constriction of the bowel, etc. Acute constipation occurs in obstruction of the intestine, paralysis of its wall as in peritonitis, and in meningitis and other conditions causing increased brain-pressure. In acute obstruction due to intussusception, ileus, etc., the frequent stools of bloody mucus but without fecal matter may deceive the doctor who does not personally inspect them.

The **consistency and form** of the normal stool vary considerably, depending on the habit and diet. Pathologically they depend on the intestinal secretion, absorption, and especially the motility. The stool may be abnormally too fluid or too solid; when very hard it is broken up into small masses resembling sheep manure, or somewhat larger masses, "scybala," which may be of even stony hardness and the size of a walnut. Such stools are common after typhoid fever and in some cases on a milk diet. These masses may in the rectum form large accumulations. When the mass is of very small caliber

<sup>5</sup> Zeitschr. f. klin. Med., 1903, Bd. 48, p. 163.

this does not necessarily indicate a stricture of the rectum, since stools of small calibre are common in conditions of anal tenesmus and of inanition, and in various nervous diseases. Boas believes that homogeneous, thick, pasty or curd-like stools in which float small cylinders of fecal matter about the size of the little finger do suggest stenosis of the lower bowel, provided they are from a patient who usually evacuates such stools.

The fæces are abnormally soft if they contain an excess of water or fat, fruit or vegetable matter. An abnormal water-content may mean unnaturally rapid peristalsis of the colon, which precludes the normal drying of the stool; or an abnormal secretion of water by the colon, as in metallic poisoning, cholera, etc.; or a disturbance of absorption from the colon. For the occurrence of fatty stools and of stools with excessive mucus, see pp. 387, 406. The vegetable foods which effect the softness of the stool are cabbage, pears, apples, plums, etc. An easy way to determine whether its softness is due to fat or to water is to press a cover-glass down hard on a small portion of the stool. If when the pressure is relieved the cover-glass springs back a little as air rushes in beneath it from all sides, much water is present; if it stays as pressed, the softness is due to fat.

Frothy stools indicate intense fermentation. They often appear acholic, since their pigment has been reduced by the organisms of decomposition.

**Color.**—The dark color of the normal stool is due to hydrobilirubin. Except in the case of nursing children bilirubin is never present in the normal stool. The longer a stool remains in the bowel, as in constipation, and the longer it is exposed to the air, the darker its color becomes. Certain foods influence the color. Milk makes stools light; meat, dark; cocoa, reddish brown; wines, dark; foods containing chlorophyll, greenish. Several drugs also influence their color. Calomel sometimes makes them green (biliverdin); bismuth subnitrite, black (bismuth suboxide); senna, santonin, gamboge, and rhubarb, yellow; while the stool containing iron turns dark, even black, after it has stood in the air. The stool dark because of digested blood is dark when first evacuated.

A clay-like appearance of the stool may be due to the amount of fat it contains, which masks its pigment; to its dilution (as in diarrhoea); to the action of the organisms of putrefaction, which reduce the bile pigments to colorless derivations; or, most important, to the absence of bile in the intestine (as in obstructive jaundice). If the paleness is due to an excess of fat, extraction of the stool with alcohol and ether will demonstrate the presence of bile; if it is due to putrefaction, the color will be restored by exposure to air, and the passage of such stools will cease after a dose of calomel. Stools

free from bile, or "acholic" stools are a greyish white, have a bad odor, and contain much fat.

Bilirubin during its passage through the bowel is so completely reduced to hydrobilirubin that under normal conditions practically none of it (except possibly minute traces in vegetable or soapy masses) reaches the cæcum or the ascending colon. The stools will contain bilirubin, however, in cases of diarrhoea due to a peristalsis so rapid that the usual reduction cannot occur, and, the higher up in the bowel the disturbance begins, the more bilirubin there will be in the stools. Under normal conditions much of the bilirubin is absorbed from the bowel. For this reason, in cases of abnormally rapid peristalsis and in cases in which from any cause absorption is disturbed the amount of bilirubin which appears in the stool will seem unusually great. This is often seen in simple diarrhoea. The amount of bile pigments in the stools is increased, even to 400 mg. in 24 hours, also in those conditions in which abnormal amounts of them are contained in the bile, as in family jaundice.\*

The presence of bilirubin in the stool does not necessarily mean trouble in the ileum, since normally considerable of this pigment does reach the ileo-cæcal valve, and this will be passed in cases of simple colitis.

Stools containing much bilirubin (and biliverdin) are intensely yellow or green and give a beautiful Gmelin's test. In Prof. Fr. Müller's clinic was a young man with obstructive jaundice. One morning he evacuated a large, soft stool the color of which was a deep golden yellow. This stool gave an intense Gmelin reaction. The obstruction in the bile-duct must suddenly have been relieved and a large amount of bile allowed to escape.

Since in the great majority of cases the presence of bilirubin in the stools must be determined by microscopic examination, Schmidt's test is recommended.

About 2 or 3 cc. of the fresh stool, consisting of selected particles representing as many as possible of its diverse constituents, are covered in a porcelain dish with a saturated aqueous solution of  $\text{HgCl}_2$  (only the pure salt should be used) and are then ground fine with a pestle, so that the mercuric bichloride will mix thoroughly with the stool. The reaction of the mixture should be acid. The dish is then covered and allowed to stand twenty-four hours, at the end of which the fragments are examined macroscopically and microscopically. The particles stained with bilirubin will have turned green, those with hydrobilirubin, red. The green masses containing chlorophyll must be excluded by microscopic examination. In diagnosis the strands of mucus which in this test are stained green with bilirubin are most important. If large, they are probably from the colon; if small

\* Tileston and Griffin, Am. Jour. of Med. Sci., June, 1910.

probably from the colon; if small the source may be the small intestine, especially if the stool be fluid. The source is surely the small intestine if the mucus contains many nuclei of cells the protoplasm of which is digested, or cells represented by fat droplets or bilirubin granules. The question is somewhat different in the case of bile-stained muscle-fibres or connective tissue and fat masses, since all in the small intestine are normally stained with bilirubin; hence their presence in the stool may mean trouble only in the colon, either too rapid peristalsis, or catarrh; to suggest trouble in the small intestine the above-mentioned masses of mucus also should be present.

Schlesinger<sup>6</sup> considers his test for urobilin (hydrobilirubin also) very delicate. The stool, if very fatty, is extracted with ether and then with acid alcohol. The reaction of the extract is made less acid with ammonia, an equal amount of zinc acetate solution (1 per cent. in absolute alcohol) added, and filtered. The filtrate gives a good fluorescence and spectrum.

Bile acids are normally reabsorbed, and hence do not appear in the stools.

**Fatty Stools.**—There is always some fat in the stools, providing there is much in the food. This may be as neutral fat, fatty acids, or soaps. The more difficultly melting neutral fats are present usually as white or yellow scales or droplets, according to their melting-point.

Fatty acids are usually in short, delicate, curved needles, and occur in thick masses, so that the shape of the individual crystal is often very difficult to make out. The soaps, on the other hand, occur in long needles which are arranged in clusters or fans, or in short plump crystals, or scales. The droplets of neutral fat are soluble in ether, the fatty acids are dissolved on warming and in ether, while the soaps are not dissolved on warming, nor are they soluble in ether unless they have been first split by acid. A test for neutral fat which is easily made is to mix the specimen under the microscope with one drop of concentrated alcoholic solution of Sudan III. which has been filtered just before using. The droplets take an orange to a blood-red color, while the soaps and the fatty acid crystals remain unstained. The students attempt to tell from crystal form alone whether it is soap or fatty acid crystals they see. A most instructive exercise is to give them for study two portions of the same stool, the one of which has been extracted with ether (but no acid added), and they see at once that the needle crystals are chiefly soaps.

Acholic stools usually contain much fat in crystals which are mixed homogeneously with the fecal matter. Such stools have a glistening gray appearance, and microscopically contain large num-

<sup>6</sup> Deutsch. med. Wochenschr., 1903, p. 561.



bers of fat droplets and large masses of fatty acid crystals which are very pretty to see.

In diarrhoea the masses of fat needles are present as minute points which may be seen with the naked eye.

Sometimes the clumps of fat are of a whitish-gray or a yellowish color like tallow, even the size of a nut; or the fat may be present as a melted oil which hardens over the stool or on the walls of the vessel containing it. The whole stool may resemble oil. In a recent case of supposed cancer of the pancreas the stool could not by appearance be distinguished from a mass of vaseline.

Such stools occur when there is an over-supply of fat ingested, hence especially in the olive oil treatment for gall-stones, in which case the lumps may vary from the size of a pea to that of a hazelnut. A much smaller amount of fat may be conspicuous in small

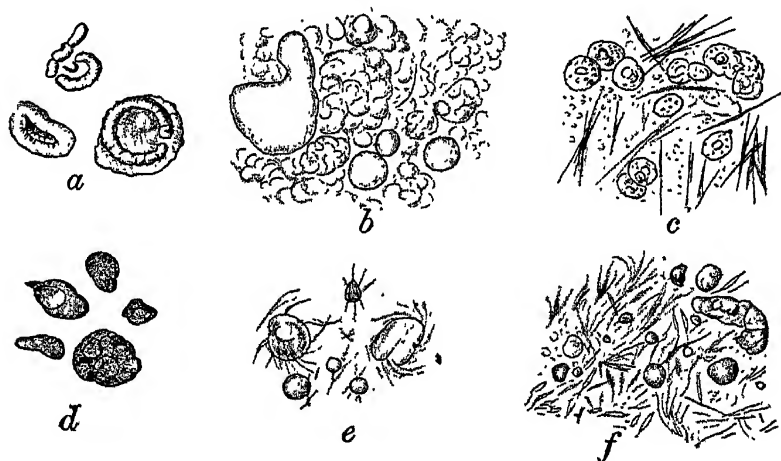


FIG. 65.—Forms of fats and soaps in stools (Schmidt and Strassburger). *a*, soaps; *b*, casein, and fat globules; *c*, fatty acid needles and leucocytes; *d*, yellow calcium soap; *e*, fatty acid crystals projecting from fat droplets; *f*, fatty acid and soap needles and scales from an acholic stool.

masses after a meal containing fats with a high melting point, as pork, mutton, or tallow.

Fatty stools are present when the mucosa or the lymphatics cannot absorb the fat: as in atrophy of the mucosa; amyloid disease; in all cases with extensive caseation of the retroperitoneal lymph glands,—*tabes mesenterica*,—the most common cause of fatty stools without jaundice, and in fact in a doubtful abdominal case the diagnosis of this condition is suggested by the stools alone; in peritonitis; and even in simple catarrh preventing absorption. There is a fat diarrhoea due to various diseases of the small intestine which should be distinguished from “diarrhoea pancreatica.”

When bile is absent, from 55 to 78 per cent. of the fat will be in stools, instead of as normally from 6 to 10 per cent. Acholic stools in cases without jaundice are particularly interesting and may contain

large amounts of fat. The cause of this condition is problematical. Some consider that there is a cessation of bile secretion; others that in all such cases bile pigment was present but has been changed to the colorless forms.

In pancreatic disease fatty stools are common, yet to be of value the stool must be very fatty. Pancreatic disease without them occurs since fat is well used if already emulsified, although in such cases the fat is insufficiently split. Müller showed that while 84 per cent. of the fat

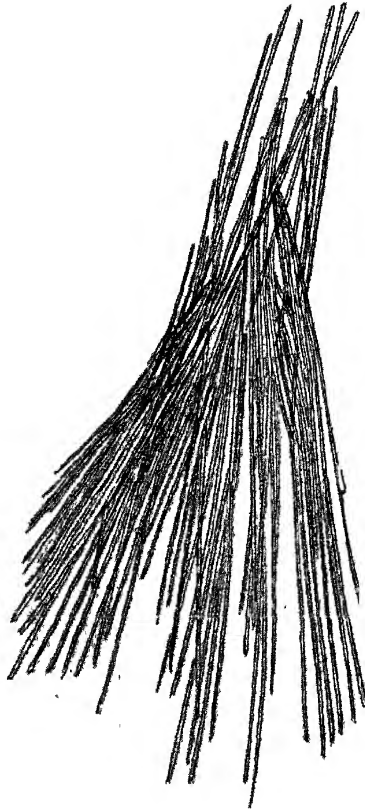


FIG. 66.—A sheaf of huge fatty acid crystals seen often in stools after they have stood a little time.  $\times 400$ .

was normally split, if the pancreatic juice be excluded only about 40 per cent. Others have found more even 80 per cent. split, yet as fatty acid not as soap. The diagnosis of pancreatic disease is exceedingly difficult, it cannot be made from the fatty stool alone, and yet if all the elements of Le Nobel's symptom complex are present it should be easy;—no jaundice, glycosuria, much fat in the stools, many fatty acid crystals but no soaps, no hydrogen sulphide, skatol, indol, etc., the stools rancid but not putrid, and with few bacteria; if all these elements are present one is very safe in assuming some pancreatic trouble.

**ESTIMATION OF FATS AND SOAPS.**—The stool is first evaporated over the water-bath until of a semisolid consistency. It is then mixed with about 50 cc. of absolute alcohol, again evaporated, and this repeated until perfectly dry. Never try to dry down a stool without alcohol. A certain amount is powdered, dried at 100° C., and then weighed. It is then rubbed up with sand and extracted from eight to ten hours with ether. The ether residue is washed with warm water. It consists of neutral fats and the fatty acids. This is dried in a desiccator and weighed.

The neutral fat may be isolated and weighed by dissolving the residue again in ether and shaking it out with a dilute soda solution, which removes the fatty acids.

The amount of fatty acids is determined as follows: A weighed amount of the ether residue is dissolved in alcohol and ether and then titrated against alcoholic solution of potassium hydroxide, phenolphthalein used as indicator.

For the soaps, the fæces already extracted with ether are boiled with acid alcohol, dried, extracted again with ether, and the free fatty acid titrated. If one wishes to determine at once neutral fats and the split fats and soap, the stool is first boiled with acid alcohol and then extracted (Müller).

In fat determinations the following values are used:

(1) The *acid value*, that is, the number of milligrammes of potassium hydroxide necessary to neutralize the free fatty acid split from one gramme of fat. A weighed amount of fat is dissolved in alcohol and ether and titrated with tenth-normal KOH, phenolphthalein used as indicator.

(2) *Köttendorfer's value*, the "saponifying value," that is, the number of milligrammes of potassium hydrate necessary to neutralize the fatty acid split off from one gramme of fat by saponification. A weighed amount of fat, 1 to 2 gms., is boiled with 10 cc. of half-normal KOH and 50 cc. of alcohol in a flask for a quarter of an hour on the water-bath, and titrated with half-normal acid, using phenolphthalein as indicator.

*Hehner's value* is the amount of fatty acid insoluble in water, which can be obtained by the saponification of 100 gms. of fat. One saponifies a weighed amount of fat, evaporates the alcohol, and treats the watery solution of the residue with hydrochloric acid. The free fatty acid is treated with boiling water, dried, and weighed.

The *Reichert-Meißl value*, that is, the number of cubic centimetres of tenth-normal potassium hydrate necessary to neutralize the volatile fatty acids obtained by the saponification of 5 gms. of fat. One saponifies a weighed amount of fat, acidulated with sulphuric acid, distils the volatile acid, and determines in the distillate the amount by titration with tenth-normal KOH (Thierfelder).

**Mucus.**—The stools always contain a certain amount of mucus (Boas). This is seldom seen even microscopically, and must be tested for chemically; any amount of visible mucus is somewhat abnormal. The mucus present is pure mucin, hence clouded by acetic acid. If rubbed up with water, an equal amount of lime water added, left to stand for several hours and then acetic acid be added, a cloud will indicate mucus.

Mucus is increased physiologically as the result of hypersecretion. This forms a glassy or cloudy coating over hard fecal masses, and evidently serves to protect the mucous membrane. It is poor in cells. It may also be present after an active purge.

That from the small intestine is intimately mixed with the stool and hard to isolate. In diarrhoea these small flecks can be picked out with a needle, and if the stool be solid they can be found as shreds or

lumps which never are bile stained. Such small flakes resemble gastric mucus. They are rich in cells and detritus of digestion, hence are not transparent. The bodies of the cells are often well digested. Some masses contain no cells but bilirubin granules and crystals in a cellular arrangement, as if the cells had been digested. The so-called "sago granules," or "spawn-like masses" of Virchow, Boas thinks are very rare. Mucus may be seen microscopically as small transparent lines and masses in the stool. The minute mucous granules or "islands" of yellowish or greenish mucus stained with bilirubin emphasized by Nothnagel as indicating catarrh of the small intestine Boas and Schmidt consider exceedingly rare, and for the most part albuminous matter rather than mucus. Much mucus is present in cancer of the rectum with stenosis (see also page 438).

If the stool consists chiefly or entirely of mucus, it is glistening and jelly-like and is evacuated in masses resembling "frogs' eggs," or in strips sometimes over a foot long suggesting to the uninitiated tapeworms or pieces of bowel. This mucus comes from the large bowel, especially the sigmoid flexure, and its passage is often preceded by a colic which is sometimes severe. Some writers have attempted to distinguish between enteritis membranacea (a mildly inflammatory condition) and mucous colitis (a secretory neurosis), but most make no such distinction. The condition is probably, except in cases where a pelvic tumor is pressing against the rectum, a secretory neurosis. Over 80 per cent. of the patients are women, most of whom give a history of constipation continuing for several years. Some pass mucous stools daily for a week, some pass one a week or one a month. others pass them still more seldom. The relation between these stools and intestinal sand is interesting (see page 417).

**Blood.**—It is of course necessary to exclude that from raw meat, and hemorrhage from the mouth, nose, lungs, and vagina. The blood may be suspected from the red or tarry black color of the stool, or found microscopically, or require chemical tests. The arrangement is important: fresh blood covering a formed stool indicates hemorrhoids; if evenly distributed with the food matter, it indicates hemorrhage into the stomach or upper bowel, providing the stool is solid. Tarry blood is usually from the stomach and duodenum; blood from small intestine (as in typhoid fever) is usually red. The blood and serum passed without fecal matter in intussusception and volvulus are important in diagnosis. Traces of blood are continuously present in the stools of patients with malignant diseases located anywhere along the alimentary tract; they are present for definitely limited, usually short, periods in cases with peptic ulcers; they are seldom found in cases with tuberculous ulcers. After a hemorrhage in typhoid fever the test may be detected chemically in the movement

which precedes the bloody stool. In chronic passive congestion blood is usually present in the stools, but not so often in cirrhosis of the liver with portal obstruction. In some cases of gastric hyperacidity also it is present, and in poisoning by mercury. The tests for occult bleeding have their greatest value in the diagnosis of malignant diseases of the alimentary tract, peptic or duodenal ulcer, and nervous gastralgia.

**GUAIAC TEST.**—About 3 gms. of fæces are made fluid by mixing them thoroughly with a little water. Then is added about one-third of their volume of glacial acetic acid. This acid fluid stool is then extracted with 10 cc. of ether. To avoid emulsifying the mixture, the tube should be slowly inverted, not shaken.

A piece of clear brown gum guaiac (any green portions should be trimmed off and not used) about the size of a cherry is crushed, and dissolved in a test-tube half full of alcohol. This tincture should assume a light-cherry color.

To about 5 cc. of the extract of the stool are added about 0.5 cc. of the guaiac tincture and then 1 cc. of commercial (3 per cent.) hydrogen peroxide, or an equal amount of old oil of turpentine. Fatty stools should first be extracted with ether to remove the fat. In the presence of blood a blue color quickly develops, which deepens and in a few minutes gradually fades to a pale green.

After the ingestion of raw meat this test of the stools may be positive, but the ordinary meat diet seldom, if ever, gives a positive result. Eggs never disturb the test. Since an acid-ether extract of the stool is used, most of the disturbing factors are eliminated, such as milk, pus, saliva, spices, and all drugs containing iron.

**THE ALOIN TEST** of Klinge and Shaer is very delicate. Preliminary to this test Koziczowsky<sup>8</sup> advises that all foods containing hæmoglobin and chlorophyll and that all drugs be discontinued. The patient is put on a milk, bread, eggs, and fruit diet. Much fat is avoided and the diet period limited by charcoal, not carmine.

The stool if very dark in color is rubbed up with ten volumes of alcohol, and this filtered off to remove the urobilin. The stool is dried on the filter paper. About 5 gms. are digested one or two minutes with 5 cc. of glacial acetic acid, then all fat extracted with 10 cc. of ether. From 1 to 1.5 cc. of oxygenated turpentine are then superimposed and 0.5 cc. of fresh 3 per cent. aloin solution (0.3 gm. aloin powdered is dissolved in 10 cc. of 60 to 70 per cent. alcohol). At the line of separation is seen in from three to five minutes a fine red ring. This means the presence of blood if the patient has been on the above-mentioned diet for some days and if the test is positive on several examinations.

<sup>8</sup> Deutsch. med. Wochenschr., 1904, No. 33.

**BENZIDIN TEST.**—Method of Schlesinger and Holst. A piece of fæces about the size of a pea is stirred up with a clean glass rod in a test-tube about one-fifth full of water. The mixture is then brought to the boiling-point over the free flame, in order to destroy any enzyme present. While this is cooling an approximately saturated fresh solution of benzidin is made by dissolving a knife-point full of benzidin purissimum (Merck) in about 2 cc. of glacial acetic acid in a clean test-tube. One then pours into a clean test-tube 10 or 12 drops of the fresh benzidin solution and from  $2\frac{1}{2}$  to 3 cc. of commercial hydrogen peroxide (3 per cent.  $H_2O_2$ ). The tube is lightly shaken. No green or blue tint should appear in the mixture of reagents. If it does, either the reagents were impure, or the tube dirty. A few drops of the boiled fæces suspension are next added. In the presence of blood, within two minutes a beautiful green, bluish green, or blue color will appear. The depth of the blue and the rapidity with which it appears will depend on the amount of blood present. Later the color will change to a violet. Only a green or blue color is positive. Slightly positive tests are not important. A little practice will soon teach what degree of color change indicates occult blood.

This test for blood is by far the most sensitive of all. It takes less than five minutes, and when negative excludes the presence of even minute traces of blood. Two or three pieces of the same solid stool, however, should be used, as one piece may contain considerable blood, another none. If positive, this test may be confirmed by the guaiac test, which is a safer one than benzidin where meat has not been excluded from the diet.

Among the recent studies on tests for blood are those of Dewis\* and Goodman.†

**Pus.**—Very rarely is there enough unaltered pus in the stools to be recognized macroscopically, and when there is it always indicates the rupture of an abscess (*e.g.*, appendix abscess) into the intestine. Yet the contents of even large abscesses may be passed unrecognized, so altered is the pus by digestion and decomposition. Pus cells are not recognizable microscopically if mixed with food, but they are when enclosed in masses of mucus. The few scattered pus cells seen in most mucus have no significance (although the mucus may have), since the normal intestinal mucosa contains many leucocytes which wander into the lumen of the bowel. Mucus containing an unusual number of single pus cells may mean catarrh; that containing masses of cells means ulcer or (especially if it also contains blood) cancer.

\* Boston Med. and Surg. Jour., Aug. 8, 1907, vol. clvii, p. 169.

† Am. Jour. of Med. Sci., Oct., 1907.

**Muscle and Albumin.**—Muscle-fibres occur practically always in the stools. The more they are digested the less evident is their striation, so that while some will show beautiful cross striation, in others only the longitudinal striation is seen, and others can be recognized as muscle-fibres only from their shape, size, and color. They are nearly all bile-stained. They are increased on a rich meat diet, as in diabetes, in diarrhœa, in which case the masses may be visible to the naked eye, and where there is disturbed absorption or secretion. The question whether there is a pathological increase in a solid stool is best judged from their number and appearance (size, shape, striation) microscopically. One is soon able to form a pretty definite opinion.

This condition of *lientery* (the presence of grossly visible particles of undigested food) is, of course, seen best in cases with a gastro-intestinal anastomosis. It occurs in a variety of conditions.

The presence of an abnormal amount of muscle-fibre in a fairly thick or solid stool and without diarrhœa is known as azotorrhœa, which is suggestive but in no way conclusive in the diagnosis of pancreatic disease, unless diabetes also is present.

**Milk curds and masses of coagulated albumin** are found in the stools of adults as well as infants on a pure milk diet or one containing much coagulated egg. The curds sometimes found in infants' stools deserve attention. The majority of the tough ones consist of casein, while many of the softer ones are almost pure fat.\* Soluble albumin, albumose, or peptone may be determined in the water extract by the ordinary tests. Normally they are present, but are increased in diarrhœa. If the biuret test be used, urobilin must be excluded.

**Starch.**—It is seldom that single well-preserved starch granules are seen in the stool of an adult, yet vegetable masses full of starch granules are common enough. If many well-preserved single starch granules occur it indicates some disturbance, either diarrhœa or hyperacidity. It is interesting that starch is never bile-stained. In the failure of pancreatic juice the starch is not increased, since the bacteria will break it up. Also the lack of bile causes no increase in the starch of the stool. The iodine test may be applied to indicate the extent to which the starch has been digested, a blue color indicating the unchanged granules; red, a slight digestion.

**Carbohydrates.**—To detect these Strassburger recommends the following: From 2 to 3 gms. of the dried stool (excluding mucus and lactose) are heated in a flask with 100 cc. of 2 per cent. HCl for an hour and a half (with a return cooler). It is then cooled and neutralized quite accurately with sodium hydroxide, filtered through

\* Jour. A. M. A., 1910, p. 372, and Talbot, Arch. of Pediatrics, Dec., 1909.

an asbestos filter, washed with water, and the filtrate brought to 200 cc. If necessary, it is filtered a second time. Fifty cc. of the filtrate are poured into a 300 cc. beaker and the sugar determined quantitatively. The amount determined of the grape-sugar multiplied by 0.94 equals the amount of starch originally present. Qualitatively, the stool may be boiled with water and the filtrate then tested with Trommer's or other solutions. It is best to precipitate the albumin with the acetate of lead; the lead is then removed with  $\text{CO}_2$  and the filtrate tested. It is seldom, however, that any glucose is found unless the stool be first boiled with acid.

**Ferments.**—The stool may be extracted with glycerin and the digestive power of the extract tested; or, according to Leo, fibrin added, which will absorb the pepsin. The fæces are mixed with chloroform water until they form a thin pasty mass. In this is suspended from 2 to 5 gms. of finely divided, previously boiled blood fibrin enclosed in a gauze bag. In twenty-four hours this bag is removed, the fibrin washed a number of times with water, and then tested for the ferments which have been absorbed. To test for trypsin, a little of the fibrin is placed in a 1 per cent. solution of soda in an incubator and the biuret test applied to the filtrate at the end of a few hours. For diastase a little of the fibrin is placed in a thick starch solution in the thermostat, and in a few hours its filtrate tested with dilute Lugol's for the blue color of starch. Normally, these ferments seem destroyed or absorbed in the intestine, yet all may be present in diarrhœa.

**Microscopy.**—For the microscopical examination of the stools care must be taken in the selection of fragments, since the one who searches at random will often find nothing. In the case of parasite eggs, etc., it is best to mix the stool with water and allow it to sediment, or to centrifugalize it. Mucous particles are to be chosen if protozoans are the object of search. In searching for blood it often makes considerable difference whether the right particle is taken or not.

**Epithelial Cells.**—Squamous epithelial cells are often found in mucus which covers the stool, and come from the anal region; many are present in cases of rectal cancer and of proctitis.

**Cylindrical epithelium** is the commonest form found. For this the mucus should be studied, and especially that which is obtained by lavage of the rectum and sigmoid. These cells will show all grades of degeneration, from fairly well-preserved cells, even goblet-cells, to those which are very fatty, and finally those in which all trace of the nucleus is lost. They occur especially in diarrhœa, sometimes in such numbers that the term "desquamative catarrh" is applicable.

Triple phosphate **crystals** are almost always present, and are irregularly formed, as a rule. Calcium phosphate crystals occur in the same form as in the urine. In addition, are calcium salts of still unknown acids, which are present in irregular, oval, or circular



masses, sometimes fissured, sometimes with a concentric striation. These are always bile-stained. The calcium soaps and calcium oxalate are frequently found (see Fig. 65).

Cholesterin occurs often, but rarely in typical crystal form, and must be tested for chemically. Charcot-Leyden crystals have been found in a great variety of diseases, but it is the consensus of opinion now that their presence always indicates some animal parasite, although it may be any, from the harmless oxyuris to the pernicious uncinaria. They are, indeed, a very valuable indication when they occur in large numbers (see Fig. 67).

Bismuthous oxide occurs as black irregular rhombic crystals after the use of bismuth subnitrate (see Fig. 69). Hæmatoidin crystals occur, but are rare.

Remnants of undigested food form the chief part of the picture, especially the thorn-like spines from various fruits and berries; the



FIG. 67.—Charcot-Leyden crystals from the stools.  $\times 400$ .

spiral cells, of which the veins of leaves are largely formed; the thick cellulose shell of various cells, some resembling soap masses, some parasite eggs; the elastic tissue from meats. The list is too long and varied to allow enumeration (see Figs. 69, 70).

**MACROSCOPIC EXAMINATION.** *Gall-Stones.*—To find gall-stones in the stools (and a careful search continues for fifteen days after the colic), the stools are well mixed with water and then rubbed through a sieve. Sometimes no stone is found even when the colic is typical of cholelithiasis. In such a case infection of the bile-duct and not a stone, may have caused the pain; or the stone may have remained in the ampulla of Vater without entirely closing the duct, or, after engaging in the cystic duct, it may have fallen back into the gall-bladder; or it may have disintegrated in the bowel, as soft stones do, perhaps all without a hard rind.

The size of gall-stones varies from that of a tiny conc.

that of a pigeon's egg. The single stones are usually spherical and rough, but when multiple, as is commonly the case in the gall-bladder, they have deep, smooth facettes. When fractured they usually prove to be formed in concentric layers. (Every suspicious mass in the stool should be fractured, since enteroliths and fragments of bone, as a bird's vertebræ, sometimes closely resemble a gall-stone.) Gall-stones are composed chiefly of cholesterin and the calcium salt of bilirubin (sometimes also salts of biliverdin, bilihumin, bilicyanin), and they have traces of calcium carbonate.

For analysis, the stone is dried and powdered. Unless it is first powdered, the mucous coating will prevent solution of the stones. It may then be dissolved in alcohol and ether, and the cholesterin crystallized out as the ether evaporates, or in boiling alcohol, from

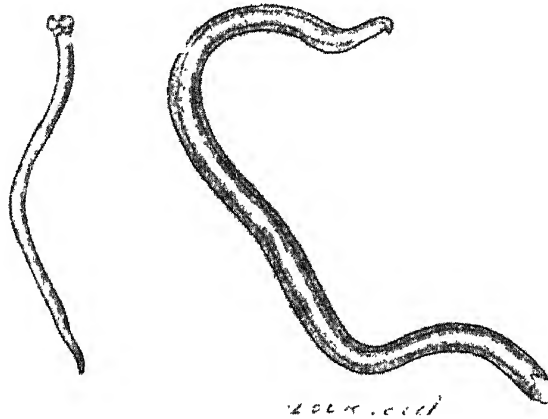


FIG. 70.—Spines forming the “down,” that on the right of a raspberry, on the left of a quince. These are often taken for the embryos of parasites.

which the cholesterin will precipitate on cooling. After the cholesterin is extracted, the residue is treated in the cold with very dilute KOH solution. This will extract the bilirubin, the yellow solution of which will give Gmelin's test. The solution will be blue if bilihumin is present.

**Pseudo Gall-stones.**—A little care would prevent the serious mistakes which result from the failure to perceive the real nature of the many concretions resembling gall-stones which are found in the stools. Every stone should be fractured and tested chemically as described above. Among the pseudo gall-stones are masses of vegetable tissue, seeds of fruits, pieces of bone, enteroliths, and masses of fats, and waxes of high melting-point. Olive oil won its reputation as a valuable means of removing gall-stones from the fact that many (even a hundred in one stool) concretions of soaps which superficially resemble gall-stones are passed after a large potation of this oil.

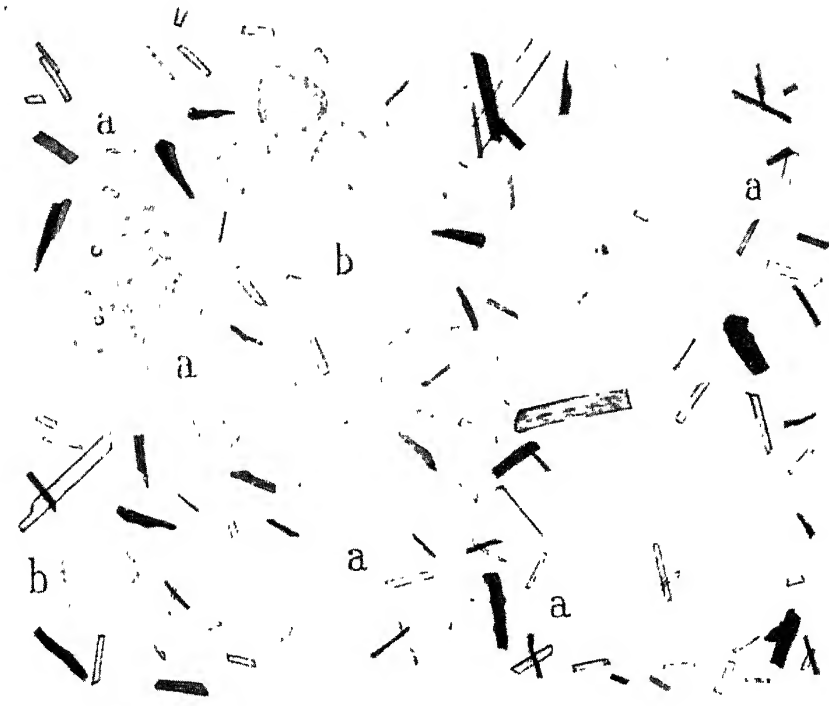


FIG. 68.—*a*, vegetable cells in stools, resembling parasite eggs; *b*, lycopodium spores; the crystals are an iron salt.  $\times 400$ .



FIG. 69.—Cells in stools. *A, B*, muscle fibers, *C, D*, vegetable cells; *E, F*, spinal fibers from a piece of lettuce; *G*, cellulose framework of vegetable tissue. The crystals are of bismuthous oxide.  $\times 400$ .



**Gall-sand.**—The sand-like concretions in which some stools abound are probably not from the gall-bladder. Genuine gall-sand would be likely to disappear in the bowel, but its failure to do so would not explain the large quantities of it in the stools (Naunyn).

**Pancreatic stones** are seldom found in the stools. If found there, they would probably occur singly. They are white and consist chiefly of calcium carbonate.

**Enteroliths.**—By enterolith is meant an incrustation of inorganic salts around a body which serves as a nucleus, usually a hard lump of food or a mass of hardened fæces. Enteroliths are seldom passed in the stools. Their chief importance is in connection with appendicitis.

**Intestinal Sand.**—Intestinal sand is small granules about the size of genuine sand, which are stony hard, which occasionally are found in the stools. Concretions over 2.5 mm. in diameter should not be called "sand." They sometimes appear in considerable quantities, even half an ounce at once. Their passage may be an incident of a nervous period and may be preceded by considerable pain. Most of the cases reported have been those of patients with neurasthenia, who also have passed mucous stools. (This is not surprising, since we do not carefully examine the stools of many normal persons.) Many cases thought to be cases of intestinal sand have proved to be instances of pseudo-sand—seeds of berries, granules from the seed case of pears (these vegetable masses can be easily recognized by studying the cross-section of a granule), concretions of altered blood pigment, bile pigment, concretions of medicines, as salol; while in other such cases the sand is real, *i.e.*, quartz swallowed with the food. The best recent article on this subject in which is emphasized the vegetable source of much that appears to be intestinal sand is that of Myer and Cook.\* They cite a case in which the granules proved to be masses containing resin and tannin, which came from the milk cells of the banana, and to which the action of the digestive fluids had given a stony hardness. But there are rare instances of a condition which apparently deserves the name "gravel-forming enteritis" (Eichorst), and this condition would seem to be a secretory neurosis.

Chemical analysis of true intestinal sand has shown that it contains phosphates and carbonates, especially of calcium, but also of magnesium, iron, etc.; while in some of the granules calcium sulphate<sup>9</sup> predominates. Practically all of them, however, contain some organic matter, many bacteria, fat, cholesterin, and urobilin. The granules are described as spherical or angular in shape, very hard, from 0.15 to 2.5 mm. in diameter, and often of a reddish-brown or

\* Am. Jour. Med. Sci., March, 1909.

<sup>9</sup> See also Garrod, Lancet, March 3, 1902, and Eichorst, Deut. Arch. f. kl. Med., 1900, Bd. 68, page 1.

green color. We have seen several cases of pseudo-sand and two very good cases of, we believe, real intestinal sand. In the one, a young boy ill with an indefinite nervous disorder, so large amounts of fine granules were occasionally passed that the sand was the most conspicuous constituent of the stool. The other patient was a young woman with an intestinal neurosis. In the latter case the granules seemed to be plugs of cells impregnated with carbonates. The nature of the dead cells could not be determined. They were the size of columnar epithelial cells. We have found a few such granules in simple diarrhoeal stools, and the further study of such stools may determine the nature of these interesting bodies.

Bedford<sup>10</sup> thinks his case shows a relationship to gout and tophus formation.

**Tumor Fragments.**—Tumor fragments and adenomatous polyps, which may occur as an independent disease or grow in the neighbor-

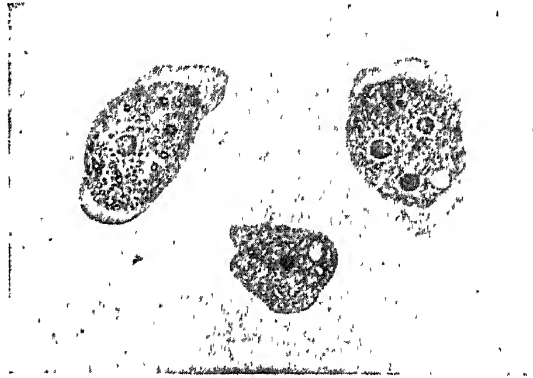


FIG. 28.—*Amœba coli* (*Entamœba dysenteriae*), common form  $\times 400$ .

hood of cancers or ulcers, may be passed in the stools, having their origin in the rectum, colon, or even higher. They are hard to recognize. If the stool is thin, these firm fragments of a grayish-red color and firm consistency may be found and the diagnosis made from the arrangement of the nuclei in the sections; but the fine details will all have been lost.

#### Intestinal Parasites. Protozoa. Rhizopoda.

**AMŒBA DYSENTERIÆ.**—This pathogenic protozoon, formerly called *Amœba coli* (and still so called by many), is now generally admitted to be the cause of "amœbic dysentery," a colitis characterized by its very chronic course a tendency to relapse, and the frequency with which it is associated with abscess of the liver. When Shiga's bacillus was first discovered, this was thought by many to be the cause of the so-called amœbic dysentery and *Amœba coli* was

<sup>10</sup> Lancet, July 26, 1902.



FIG. 72.—*Amoeba coli* (*Entamoeba dysenteriae*). An uncommon, very hyaline, and very amoeboid form of parasites, usually filled with red blood-cells. The small forms are true amoebae from a normal case *Entamoeba coli*, drawn to the same scale.  $\times 400$ .





considered a secondary invader; but now amoebic dysentery and bacillary dysentery are recognized as two distinct diseases.

Amoebæ dysenteriae are usually abundant in the masses of bloody mucus passed in the stools of patients with amoebic dysentery. But in each case most of the amoebæ are in the floors of ulcers which undermine the mucosa of the colon and of the ilium, and in the sinuous fistulae which radiate from these ulcers under the mucous membrane. They are also found in the contents of, but more easily in the walls of, the sterile liver abscesses which complicate this form of dysentery, and in the sputum of patients into whose lungs such abscesses have ruptured.

*Amoeba dysenteriae* (Fig. 71, 72) is a rhizopod which varies in diameter from 8 to 50 microns. It has a clear hyaline ectosarc, seen best in the pseudopods, and a finely granular endosarc, which usually contains some of the parasite's ingesta (red blood-cells, leucocytes, bacteria, epithelial cells, and particles of food), and often one or more vacuoles which do not pulsate. Its spherical nucleus, about 6 microns in diameter, is sometimes, especially when the ectosarc contains little foreign matter, clearly seen, but as a rule is not visible in the living parasite. To demonstrate the nucleus, one kills the organism with corrosive sublimate, or stains it by appropriate methods.

The organisms found in various cases of dysentery do not all look just alike. Those causing some cases differ so much from those causing others that the temptation is ever present to try to separate several different varieties of the parasite.

In the fresh stool, if the stage of the microscope is not too cool, this amoeba is a very actively motile organism. It may project one pseudopod, or several in different directions. If one, the parasite may retract it again, or may by flowing into it change its own position, or the pseudopod may merely flow around the periphery of the organism. The amoeba sometimes moves slowly, sometimes so fast that it is with difficulty kept in the field of the microscope.

These amoebæ multiply by simple division. They are very sensitive to an acid reaction of their environment.

Resting, resistant forms, or encysted amoebæ have been described. They are composed of divisions of nuclei and of protoplasm clumped around each new nucleus. It is probable that the infection of a new host is effected by these encysted forms.

Since it is almost impossible in examining the stool to distinguish resting amoebæ from degenerated swollen epithelial cells, it is of great importance that only cells which unmistakably project a pseudopod should be called amoebæ, and that all those the motility of which is doubtful should be discarded, no matter how closely they may re-

semble amoebæ (although quiet cells resembling amoebæ and which contain red blood-cells are probably this parasite). If one conscientiously follows this rule, that when there is the least question the object should be called an epithelial cell, he will usually be correct and save himself from many blunders.

The stools should be examined while fresh and while warm, for the parasite is sensitive to temperature. If the specimen is kept warm the parasite will remain active for even twenty-four hours. (The common mistake of overheating the stool should be avoided.) If particles of blood-stained mucus are found they should be examined; if not, any mucus; if there is no mucus, the liquid part of the stool is examined. If the stool is firm it is well to give the patient a dose of salts and examine the liquid stool which results; or a solid fragment of stool may be mixed with normal salt solution, and this fluid examined. Some prefer to pass a rectal tube and examine the little mucus which the edges of the eye of the tube will scrape from the mucosa. The parasites are usually found in clusters of scores or hundreds in islands of mucus. A warm stage is a valuable aid in keeping the parasite motile and therefore recognizable during the search, although this is almost a luxury, since the top of a radiator is often a good table on which to rest the microscope.

During an acute exacerbation of a chronic case of amoebic dysentery the patient passes five or six stools a day, seldom more. These stools are loose, not watery, and contain mucus which is usually blood-stained, and generally some free blood. During the periods of constipation which separate the periods of diarrhoea the amoebæ can often be found in the firm stools. Some cases of amoebic colitis have no periods of dysentery; their history is one of years of constipation. A rather large proportion of the cases of amoebic liver abscess have just this course. Other cases are very acute and are marked by almost continuous passing of small portions of blood-stained mucus. Still other cases are latent, causing the patient few, if any, symptoms, although the amoebæ in his stools may be numerous.

Since *Amoeba coli* was first discovered, or, more truly, before the pathogenic amoeba was described by Lösch, it was known that amoebæ are often found in the stools of persons without any dysentery or any ulcerative disease of the bowel. One finds them in various cases of diarrhoea, in typhoid fever, in acute and chronic enteritis, colitis, and proctitis, and even in the stools of healthy men. In 1893 Quincke and Roos separated: *Amoeba coli* (Lösch), 15 to 25 microns in diameter (encysted forms 10 to 15 microns), pathogenic to men and to cats; *Amoeba coli mitis*, which is 25 to 35 microns in diameter, may ingest bacteria, but never red blood-cells, and is slightly pathogenic to man, causing a mild enteritis, but not at all

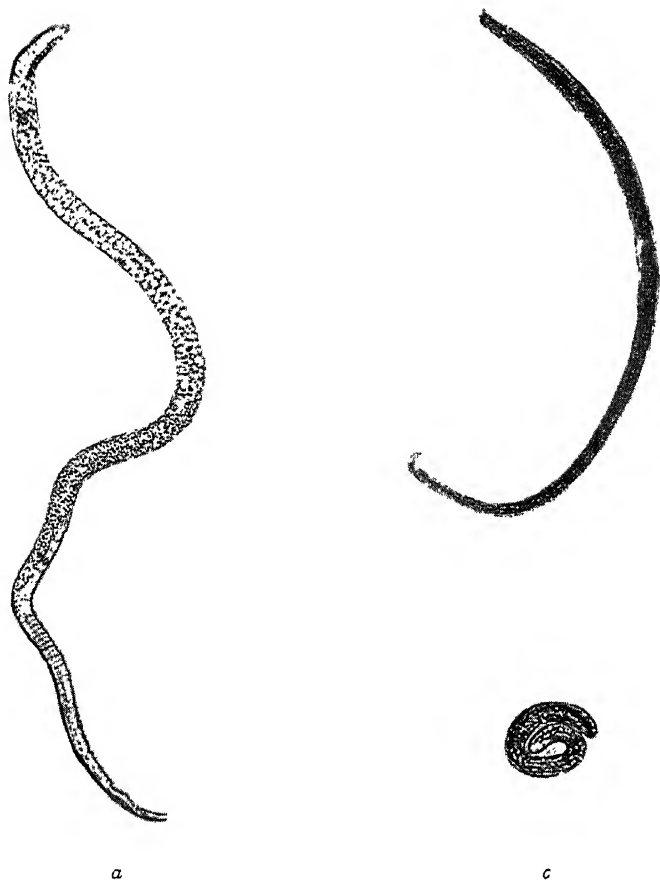


FIG. 72a.—*Trichina spiralis*. *a*, adult female. *b*, adult male.  $\times 90$ . *c*, embryo.  $\times 400$ . (I am indebted to Dr. C. L. Overlander, of Boston, for these photographs.)

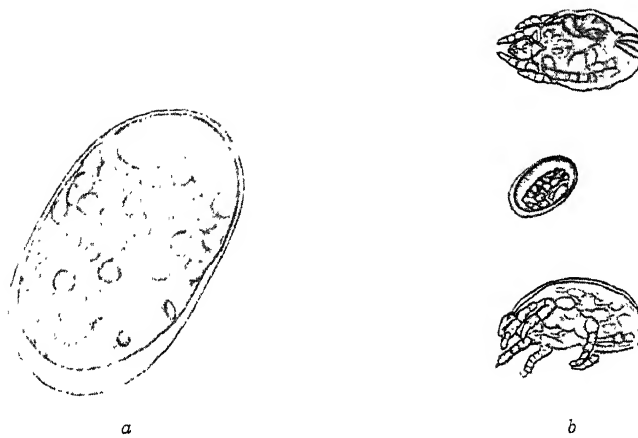


FIG. 73a.—Eggs of *Tyroglyphus siro* (cheese- or flour-mite). *a*, an egg magnified  $\times 400$  to allow a comparison of size with the eggs of Fig. 73. *b*, in the centre an egg and above and below two mites soon after they hatched and had developed somewhat.  $\times 100$ . NOTE.—We picture these eggs merely as a warning to the student that not all the eggs he may find in the stools are eggs of important parasites. One not infrequently finds eggs of the great variety of harmless insects, etc., which are swallowed with the food. When in doubt concerning an egg it should be carefully measured and then the attempt made to hatch it. If still in doubt the specimen should be sent to the Washington Laboratories (Department of Health).

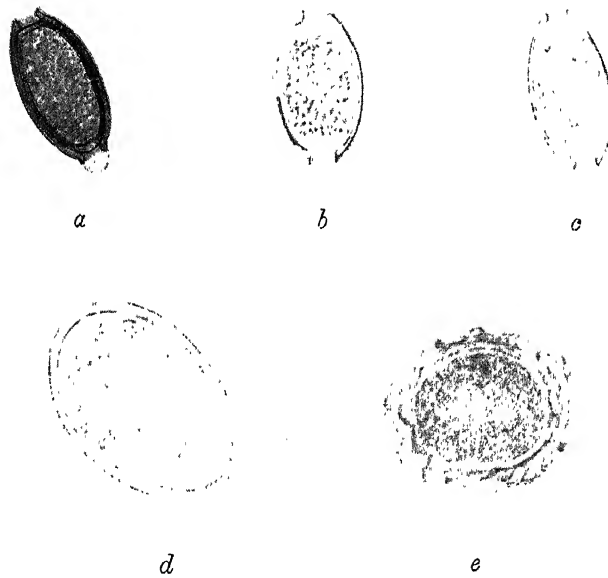


FIG. 73.—Parasite eggs in stools. *a, b, c*, eggs of *trichocephalus dispar*, showing the different colors (species?); *d, e*, *ascaris lumbricoides*; *d*, envelope lost; *e*, perfect.  $\times 400$ .



pathogenic to cats; and *Amœba intestini vulgaris*, similar in appearance to *Amœba coli*, but not at all pathogenic.

The best recent contribution to the subject is by Schaudinn,<sup>11</sup> who separates *Entamœba coli* from *Entamœba histolytica*,<sup>12</sup> the former the common harmless variety, the latter the pathogenic form causing dysentery.

*Entamœba coli* can be found in the stools of 65 per cent. of normal persons after a dose of Epsom salt (Craig). Its size averages somewhat smaller (10 to 20 microns in diameter), it is less actively motile, there is less difference between endosarc and ectosarc, the latter is less refractile, the former has less demonstrable structure, vacuoles are less common, and the nucleus more distinct than in the pathogenic variety. What is more important, the pathogenic variety shows no encysted stage, but does multiply by sporulation. (For more details, see Craig, loc. cit.)

The problem of non-pathogenic amœbæ is of interest to the zoologist, but the medical man should consider every amœba he finds in the stools as possibly pathogenic. Musgrave believes that an amœba which has been harmless may become pathogenic. Of the 300 persons in Manila whom he examined 101 were infected with amœbæ. Of these 61 had dysentery, and the other 40 had no sign of the disease. During the next five months, however, every one of these forty had a definite dysentery.

Amœbæ may be cultivated, but it is with difficulty and only with certain bacteria. These cultures withstand drying for fifteen months.

**Flagellata.**—In human parasitology the flagellata which are important are of the enflagellata, and of these the protomonadina and the polymastigina. Flagellated rhizopods and lower plants must be excluded as extraneous.

*Polymastigina.*—These are flagellata with three equal or from four to eight unequal flagella inserted at different points. They may also have an undulating membrane, often mistaken for a row of cilia. Of these are two groups of importance, the *Trichomonas* and *Lamblia*.

**TRICHOMONAS.**—This is a pear-shaped organism, rounded in front, pointed behind, with at its anterior end three to four equally long flagella which often are united at their base. The undulating membrane, which is usually present but not always seen, begins at the anterior pole and extends obliquely backward. The nucleus is anterior, and behind it are one or more vacuoles which do not pulsate. It is interesting to study the various sizes and shapes of these flagellated organisms, and their movements, particularly so when in an old specimen the flagella have been withdrawn and then evidently the attempt made to extrude them, in which case the membrane is

<sup>11</sup> Arbeit. a. d. K. Gesundheitsamte, 1903, xix, p. 563.

<sup>12</sup> Craig, Am. Med., May 27, June 3, 1905, considers the name *Entamœba dysenteriae* better.

projected to some distance in three or four different directions. The activity of these movements aids to distinguish them from amœbæ.

*TRICHOMONAS VAGINALIS* (Donné).—This parasite (Fig. 74) is from 15 to 25 microns long, from 7 to 12 broad, with its posterior end drawn to a thread, its cuticle thin, protoplasm free from granules. It has three flagella, as a rule, which sometimes seem united at base, the fourth, which is sometimes described, probably being the edge of the undulating membrane. These are of equal length. The undulating membrane extends spirally backward from the anterior pole. This parasite is found in abundance in the acid secretion of catarrhal vaginitis.

In the intestine various forms have been described under such names as *Protoryxomyces coprinarius*, *Monocercomonas hominis* (Grassi), *Cimænomonas hominis* (Grassi), *Trichomonas hominis* (Grassi), *Cercomonas coli hominis* (May), but all of these are now

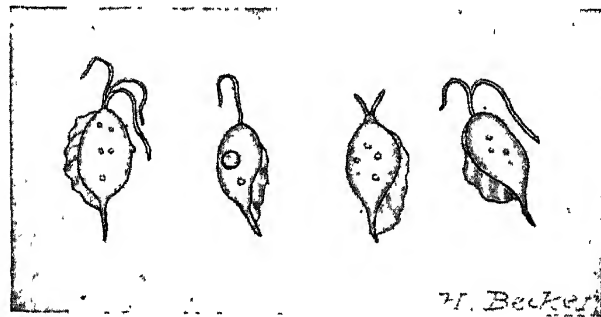


FIG. 74.—*Trichomonas vaginalis*.

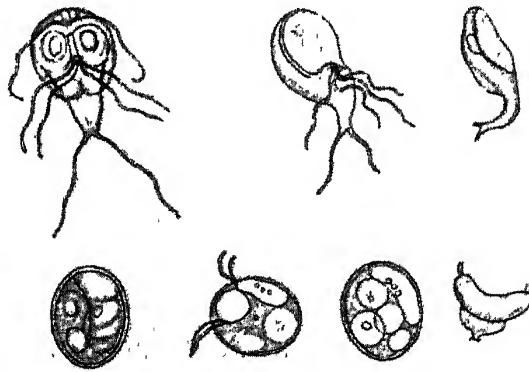
considered to be identical with above-mentioned *Trichomonas vaginalis*, which parasite can live in the vagina, the urethra, the large and the small intestine, the stomach, can even appear in the mouth, and be found in the sputum from lung cavities and in the Dietrich's plugs. It has long been a question whether these parasites were harmless or not; whether they caused a diarrhœa or merely aggravated a trouble that was already present. It is now considered pathogenic, and we could mention one or two cases in which this parasite seems to have been the cause of a severe diarrhœa.

*LAMBLIA* (Fig. 75).—This is a family of pear-shaped organisms with a deep concavity on their inferior surface and with four pairs of flagella, three on the edges of the concavity and one at its posterior extremity. Various names of this parasite are *Lambia intestinalis*, *Cercomonas intestinalis* (Lambl), *Cercomonas coli* (May), *Trichomonas intestinalis* (Leuckart).

The protoplasm is hyaline and finely granular, never containing solid inclusions, and with a very fine cell membrane. The nucleus is dumb-bell-shaped and at the base of the concavity. It has four pairs



of flagella of almost equal length (9 to 14 microns), one on each side of the concavity, two pairs at the projection at the inferior edge of the concavity, and one pair at the end. This parasite lives in the jejunum and the duodenum, and sits still on the top of a columnar cell, which it embraces with its concavity. In some cases they are found in such numbers that they form a membrane covering the mucosa. When they reach the large intestine they are encysted, and then are round or oval bodies with a very distinct membrane, within which is the folded organism. The motile parasite is thus not seen in the stools unless in a severe diarrhoea, in which case they have not had time to encyst themselves. They then move with some rapidity and very irregularly, lashing about in an aimless manner. They vary from 10 to 21 microns in length, and from 5 to 12 in width. The



*H. Becker.*

FIG. 75.—*Lamblia intestinalis*, showing the motile form in different positions, and stages of its encysting.  $\times 900$ .

encysted forms, from 10 to 14 microns long by 8 to 10 wide. The stools should be examined as fresh as possible and on a warmed stage. The number in the stools may be enormous, even estimated at eighteen millions in twenty-four hours. Their surest point in diagnosis is the concavity and the dumb-bell-shaped nucleus. The host is chiefly the mouse, rat, rabbit, dog, sheep, cat, etc. Men are evidently infected from water. They have been found principally in children. While their pathogenicity is uncertain, they may aid in the disease, and they certainly live best where there is intestinal trouble.

We have recently had a case of marked infection in a medical ward, recognized in the stool by the encysted forms. When purged with Epsom salt the motile *Lamblia* was easily found. The egg-like encysted forms were present in the fatty stools in great numbers, from five to ten being present in most of the fields (400  $\times$ ). It was

interesting to watch the organism encyst itself, first withdrawing its tail flagella, then becoming more oval, with the concavity last to disappear, from the edges of which the flagella projected until the cavity disappeared. In some cases the lines in the encysted form, commonly taken to indicate the folds of the parasite, seemed the edges of this closed cavity. A membrane could in some be distinctly seen.

*Protomonadina*, forms which have one or two equal or one principal flagellum and one or two smaller ones, are much smaller and of a lower class than the above mentioned polymastigina. Two of the three forms occur in man, *Cercomonadidæ*, which have one flagellum and no undulating membrane, and the *Trypanosomidæ* which have one flagellum and an undulating membrane which reaches the whole length of the parasite.

**CERCOMONAS HOMINIS.**—These parasites are small flagellates occurring in the stools, from 10 to 12 microns in length, but varying from 8 to 16 microns. They are pear-shaped, with a long flagellum at the anterior end which may be even twice the body-length. They move very rapidly. Their pathogenicity is doubted. They have also been found in other parts of the body, including the sputum.

**Infusoria.**—The infusoria are bilaterally symmetrical protozoans which have a permanent shape, are ciliated, contain contractile vacuoles, and usually a macro- and micro-nucleus. The order which is of most importance to us now is that of *Heterotricha*, which are uniformly ciliated, but with a border of longer cilia around the peristome, and of these, the *Balantidium* group.

**BALANTIDIUM COLI** or **PARAMÆCIUM COLI.**—These parasites (Fig. 76) are oval, covered uniformly with cilia, are from 60 to 100 microns long, from 50 to 70 broad, with the mouth at the anterior end, a funnel or cleft-shaped entrance extending one-fourth the body length and surrounded by cilia about twice as long as those over the body. The ectosarc and the endosarc are clearly differentiated. The latter is finely granular, containing many fat or mucous droplets, starch granules, even red blood-corpuscles, leucocytes, and bacteria. The nucleus is kidney- or bean-shaped and also accompanied by one or more accessory nuclei. Usually there are two contractile vacuoles which pulsate feebly. The surface is traversed by parallel longitudinal lines connecting the two poles, most distinct at the anterior end. The anal orifice is at its posterior end, which is rather blunter than the anterior. This parasite occurs especially in the colon, but in severe cases may be found even in the jejunum, and may be present

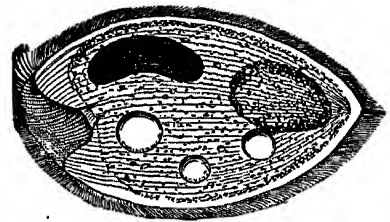


FIG. 76.—*Balantidium coli*.  
(Copied from Braun.)

in the stools in some cases in enormous numbers, even from one to two hundred in one drop. The blood-stained mucus should be examined, which also contains many epithelial cells. The pathogenicity of these parasites has been questioned, and yet it is now quite unanimously granted that they may be the cause of the most severe and stubborn catarrh, which may even be fatal. Others say they invade a catarrhal intestine as secondary parasites; others say they may set up a catarrh which continues after they die out (Henschen). Between eighty and ninety such cases are now on record, especially from Russia. A very good description is that given by Strong and Musgrave.<sup>13</sup> Klimenko<sup>14</sup> concludes that the diarrhoea may first be due to their mechanical irritation of the rectal mucosa, later to catarrhal or even ulcerative colitis; that they invade the intestinal wall, enter the blood-vessels, and sometimes cause emboli to distant organs; but that their action and effect are chiefly mechanical is shown by the absence of any degenerative or inflammatory changes which would point to a toxine.<sup>15</sup>

We have seen these parasites in a few cases of diarrhoea, but in none severe enough or in numbers great enough to attach much importance to their presence.

**Enthelmintha. TRICHINA SPIRALIS.**—The adults may be in the stools, but they have practically never been found. Adults occur in intestines of rats, pigs, dogs, and cats. The male worm is from 1.4 to 1.6 mm. long and 0.04 mm. wide, the female 3 to 4 mm. long by 0.06 mm. wide. After the encysted embryos are swallowed with meat the capsule is digested in the stomach and the embryos rapidly mature in the intestine. On the second day the males die and the females bore their way into the mucosa of the villi, or at the base of the Lieberkuhn glands, at which point they lie in the lymph spaces and viviparously hatch the young (0.09 to 0.1 mm. long, 6 microns wide) into the lymph and blood-stream. These in nine or ten days begin to take their permanent habitat in the muscles, travelling passively in the blood-stream and also actively boring their way. They are then about 1 mm. long. A capsule is formed around them, and in about one year this begins to calcify. (See Fig. 72a, page 418.)

**ASCARIS LUMBRICOIDES.**—This, the ordinary "round worm," is a common intestinal parasite, occurring in about 0.4 per cent. of all cases (Garrison, Ransom, and Stevenson). The female is from 20 to 40 cm. long, 5 mm. thick, the tail straight and conical. The male is from 15 to 25 cm. long and 3 mm. thick. The posterior end is bent ventrally into a hook, and terminates in the two spicules. The mouth of both is surrounded by three papillæ. The color of these worms is gray or a dirty reddish-brown. While it is an inhabitant of the small intestine, and hence is most commonly seen in the stools,

<sup>13</sup> Johns Hopkins Hosp. Bull., February, 1901.

<sup>14</sup> Beitr. z. path. Anat. u. allg. Path., 1903, Bd. 33, p. 281.

<sup>15</sup> Ehrnrooth, Zeitschr. f. klin. Med., 1903, vol. xlix. p. 321.

yet it is often present in the vomitus. Its eggs (Fig. 73, *d*, *e*) are found in the stools in large numbers. These are elliptical, 50 to 70 microns long and 40 to 50 microns wide. Those which we have

measured varied from 65 to 80 by 45 to 55 microns. These eggs have an unsegmented protoplasm surrounded by a thick transparent shell, which in turn is covered by a thick, gelatinous very uneven lumpy envelope, which is usually bile-stained. To find this worm it is to be recommended that santonin be given, which will have a therapeutic as well as a diagnostic value.

Smith and Goeth<sup>16</sup> consider the worm they report a new species,—“*Ascaris texana*.”

Attention has recently been called to the unfertilized eggs of *Ascaris lumbricoides*. These look so different from the fertilized ova that for a long time they were not recognized and led to error in diagnosis. Dr. O. T. Logan, who was one of the first to recognize them,\* writes me that the cell represented at the left-hand edge of Fig. 68 was certainly a typical unfertilized ascaris egg. Houghton (personal communication) in a series of fecal examinations on 500 patients in Wuhu, China, found that 71.2 per cent. were infected with this parasite. Of these 500 persons, 38.6 per cent. passed

fertilized eggs only, 29.6 per cent. fertilized and unfertilized eggs, and 3 per cent. unfertilized eggs only, the latter being usually mistaken for vegetable cells.

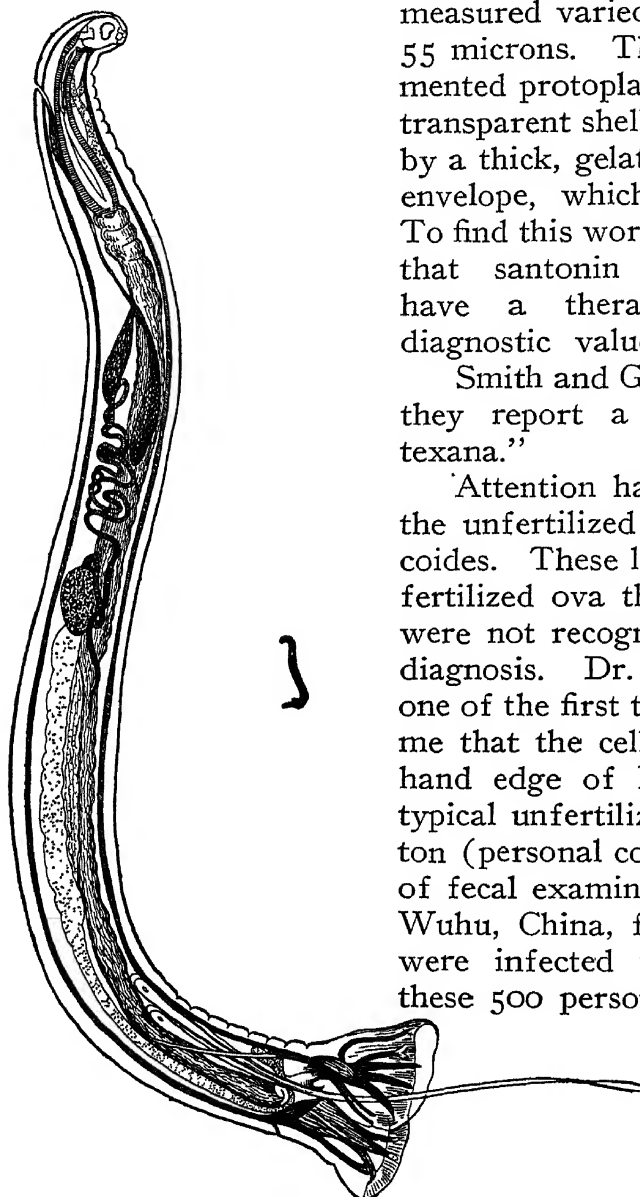


FIG. 78.—*Ankylostomum duodenale*, natural size to right, much magnified male on left. (From Braun.)

**OXYURIS VERMICULARIS.**—This little parasite (Fig. 77) occurs in the rectum and colon even as high as the cæcum where it inhabits the appendix, but it may even reach the stomach. It can travel

<sup>16</sup> Jour. Am. Med. Assoc., 1904, No. 8.

\* Rep. Am. Soc. Trop. Med., 1908.

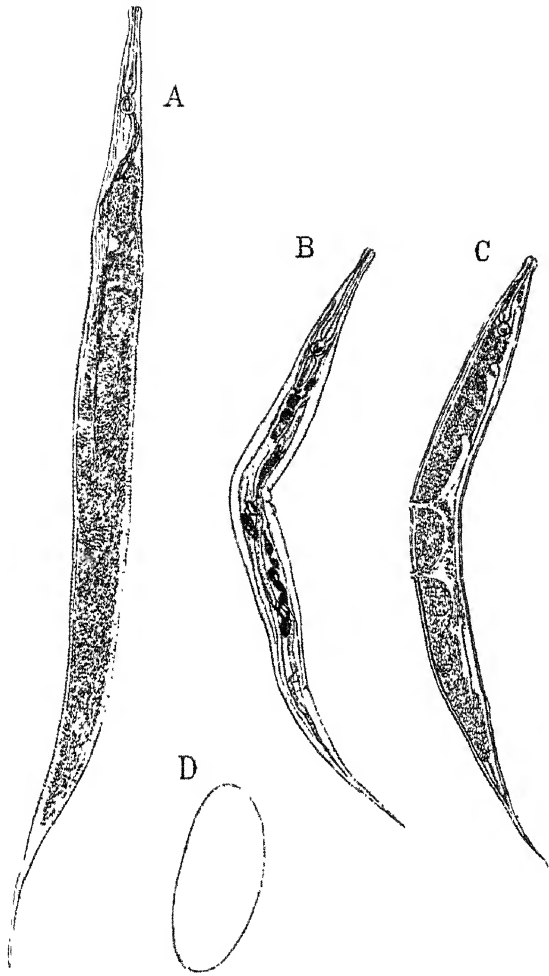


FIG. 77.—*Oxyuris vermicularis*. A, B, and C, adults; A and C are females full of eggs.  $\times 12$ .  
D, egg,  $\times 400$ .



through uterus and tube to Douglas's cul-de-sac. According to some, it can bore its way through the intestinal wall and cause an abscess. It is present in perhaps 0.8 per cent. of adults. The adult male is from 3 to 5 mm. long, with its posterior end bent into a ventral hook. The female is 10 mm. long and 0.06 mm. wide. They are white in color. Their eggs are 50 microns long and 16 to 20 microns wide, and have a characteristic asymmetry. The parasite leaves the rectum to lay its eggs on the skin surrounding the anus, at which time the itching occurs. The eggs when deposited already contain a well-developed embryo. It is rare to find the eggs in the stools, except in the mucus which the stool gains on passing through the lower rectum, hence the skin around the anus should be

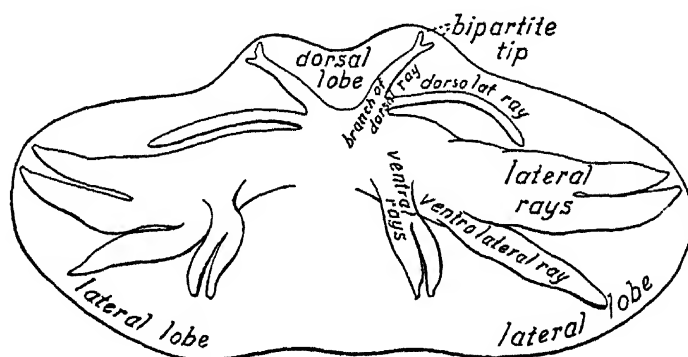


FIG. 79.—Caudal bursa of *Uncinaria americana*. (Schematic.)

examined for the adults. It is only accidentally that eggs are found except by scraping the surface epithelium from the margin of the anus, and this is the best method.

Very good plates of the development of this worm are given by Heller.<sup>17</sup>

*Uncinaria Duodenalis* and *Uncinaria Americana*.—These two parasites (Figs. 78-84), belonging to the nematode family, Strongyloidæ, are the cause of some of our severest anæmias. They have recently attained great importance in this country through the demonstration by Stiles that they are the common cause of the "anæmia of the South." In five hundred cases chosen at random they were present in 3 per cent.<sup>18</sup> They have long been known in their connection with bricklayer's anæmia, tunnel-workers' anæmia, Egyptian chlorosis, miners' anæmia, etc. The best description of these parasites is that given by Stiles in the Eighteenth Annual Report of the Bureau of Animal Industry, 1901.

UNCINARIA DUODENALIS, ANKYLOSTOMUM DUODENALE.—The body is cylindrical, somewhat attenuated anteriorly. The buccal

<sup>17</sup> Deutsch. Arch. f. klin. Med., 1903, Bd 77, p 21

<sup>18</sup> See also Smith, Am. Jour. Med. Sci., 1903, vol. cxxvi.

cavity (Fig. 82) has two pairs of ventral teeth curved like a hook and one pair of dorsal teeth directed forward; the dorsal rib does not project into the cavity. The male is from 8 to 11 mm. long with a caudal bursa (Fig. 80) with dorso-median lobe, and prominent lateral lobes united by a ventral lobe. The dorsal ray divides at a point

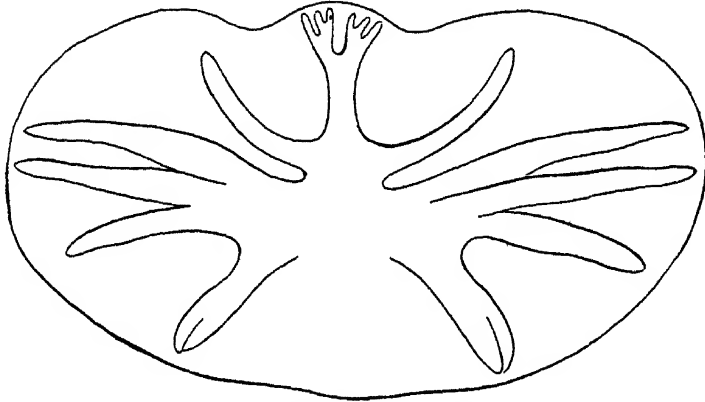


FIG. 80.—Caudal bursa of *Uncinaria duodenalis*. (Schematic.)

two-thirds its length from the base, each branch being tridigitate. The spicules are long and slender. The female is from 10 to 18 mm. long, the vulva at or near the posterior third of the body. The eggs are ellipsoid, 52 by 33 microns, laid in segmentation. Development is direct without intermediate host.

*UNCINARIA AMERICANA* (Stiles, 1902).—This differs from the

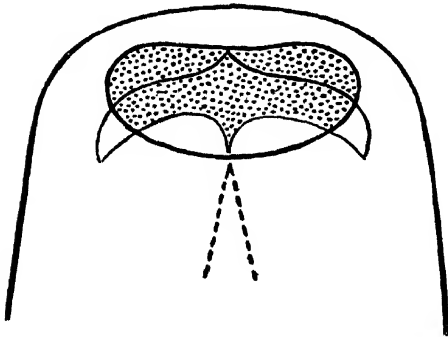


FIG. 81.—Head of *Uncinaria americana*. (Schematic.)

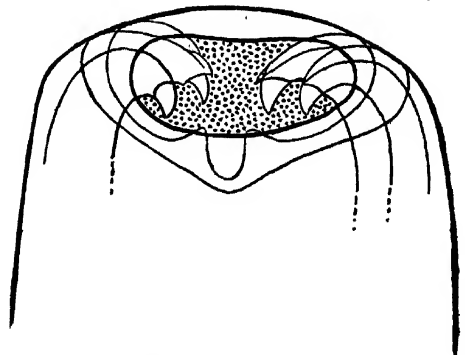


FIG. 82.—Head of *Uncinaria duodenalis*. (Schematic.)

above-mentioned form in that its buccal cavity (Fig. 81) has a dorsal pair of prominent semilunar plates or lips, and a ventral pair of slightly developed lips of the same nature, no hook-like teeth. The dorsal conical median tooth projects prominently into the buccal cavity. The male is from 7 to 9 mm. long, the caudal bursa (Fig. 79) with a short dorso-median lobe, which often appears as if divided into two lobes, and with prominent lateral lobes united



laterally by an indistinct ventral lobe. The common base of the dorsal and dorso-lateral rays is very short. The dorsal ray is divided to its base, its two branches being prominently divergent and their tips bipartite. The spicules are long and slender. The female is 9 to 11 mm. long, the vulva in the anterior half of the body but near the equator. The eggs are ellipsoid, 64 to 72 by 36 to 40 microns,

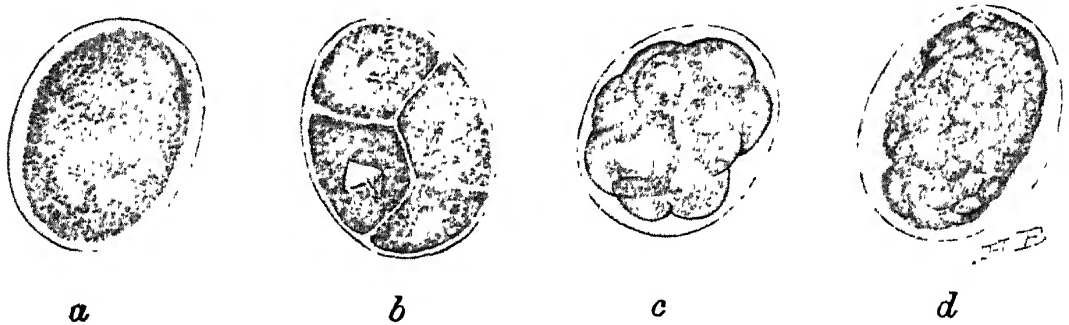


FIG. 83.—Eggs of *Uncinaria duodenalis*. *a*, unsegmented; *b*, with four segments and showing nuclear spindles; *c* and *d*, later stages of segmentation.  $\times 400$ .

in some cases partially segmented in utero, in other cases containing a fully developed embryo when laid.

The eggs (Fig. 83) of the *Uncinaria* worms are found in the stools either unsegmented or during the early stages of segmentation. They have a thin clear shell. While the yolk will show all stages of segmentation, it is rare to find eggs with an undivided yolk, those

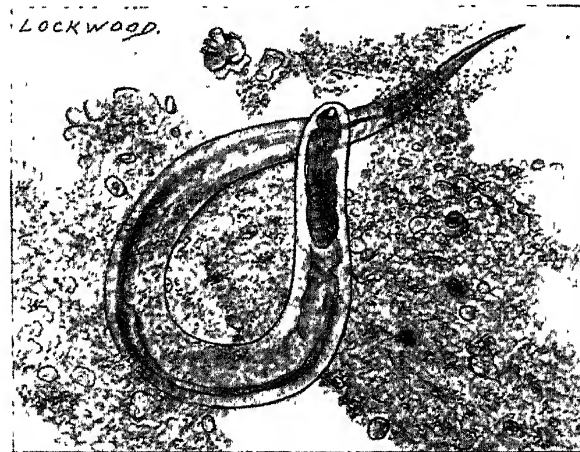


FIG. 84.—Embryo of *Uncinaria* (*americana*?) found in the stool.  $\times 400$ .

divided into four, eight, sixteen, or more segments being the most common. The eggs should be searched for in the fæces, a small amount being mixed in a drop of water and spread on the slide. The older the fæces and the warmer the weather the more advanced will

the segmentation be. This is very true of *Uncinaria americana*.

To find uncinaria eggs in stools, where they are not numerous, it is well to follow the suggestions of Pepper \* and those of Dock and Bass.† The stool is diluted with about ten volumes of water, is strained through two or three layers of gauze in a funnel, and is then centrifugalized until the sediment is just thrown down. The supernatant fluid is poured off, more water is added, the tube is well shaken, and the stool is again centrifugalized. Since uncinaria eggs stick to glass in a peculiar way, a drop of the sediment is put on a glass slide, and the slide is gently immersed in water, which will wash off much of the sediment, while the uncinaria eggs will stick to the glass. Another drop of the sediment is then put on the

same spot, and the immersion is repeated. The process is repeated several times. In this way one may obtain specimens abounding in eggs. One disadvantage in the method is that eggs of other parasites are lost. The adults may be found in the sedimented stool after a small dose of thymol followed by oil. The adults are usually red from the blood with which they are filled. They occur in the duodenum, jejunum, and ileum, many thousands sometimes in one person, although, as a rule, not more than a few hundred. While they do not multiply in the intestine they may live there for five years.

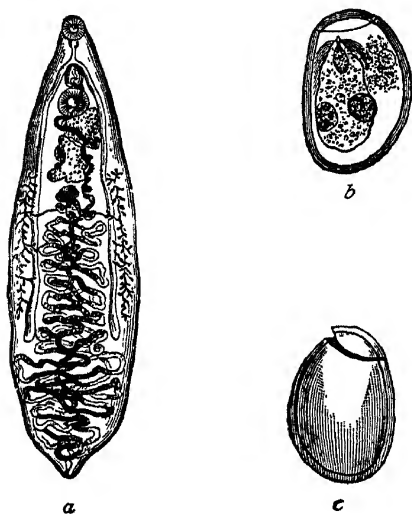


FIG. 85.—*Distoma lanceolatum*. a, adult; b, egg with embryo; c, empty shell. (From v. Jaksch.)

TRICHOCEPHALUS TRICHIURIS. TRICHOCEPHALUS DISPAR. TRICHIURIS TRICHIURIA.—This is the ordinary whip-worm, a worm from 4 to 5 cm. long, with two-thirds of the length a whip-like tail. They occur especially in the cæcum, but also in the colon; rarely in the small intestine. They are perhaps one of the most common of intestinal parasites, in 10.3 per cent. of adults in this country, but in the stools of 45 per cent. in some parts of Germany, and at autopsy in 100 per cent. in Southern Italy. Their eggs (Fig. 73) are very characteristic, being from 50 to 54 microns long and 23 microns wide, with an unsegmented yolk and a very thick shell, into each pole of which is inserted a plug. These eggs present an interesting variety of colors, some being light lemon-yellow, some deep yellow and some a dark brown. In the stools we have also found eggs which were certainly those of this worm, but which had no

\* The Jour. of Med. Research, March, 1908, vol. xviii, No. 1, p. 75.

† Hookworm Disease, Mosby Co., St. Louis, 1910.

plugs at the end. These may be very young eggs, since they have this shape. This parasite is harmless as a rule, but may cause enteritis and the severest and even fatal anæmia. In a recent review of the effect of this worm, Becker<sup>19</sup> classifies the symptoms as gastrointestinal, diarrhœa due to ulcers or catarrh, blood in the stools, symptoms of appendicitis even; nervous symptoms simulating meningitis (Erin thought beriberi due sometimes to this worm); and anæmia with all its symptoms.

**STRONGYLOIDES INTESTINALIS.**—*Anguillula stercoralis* et *intestinalis*; *Leptodera stercoralis* et *intestinalis*; *Rhabditis stercoralis*, *Rhabdomena strongyloides*, are a few of the many synonyms. The rhabditiform larvæ of this parasite found in the stools measure from 0.3 to 0.6 mm. long and from 16 to 22 microns wide. They are in very active motion. The best way to find them is to make a depression in the fecal mass, fill it with water, place the stool then in a thermostat,

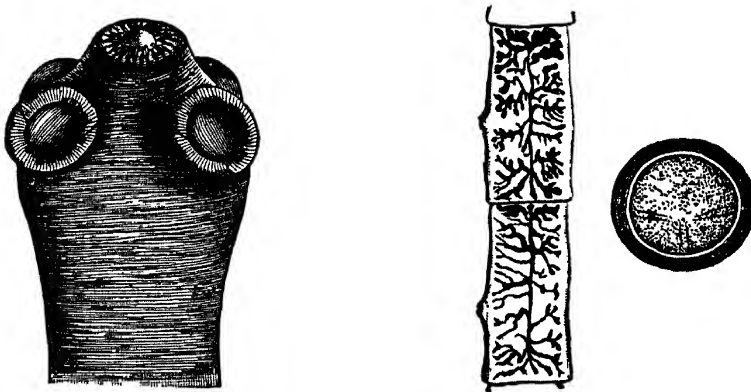


FIG. 86.—*Tænia solium*: Head (magnified), proglottis (actual size), and egg (magnified). (Zeiss's eye-piece IV., objective IV.) (From a preparation by Cori and v. Jaksch.)

and examine a drop of this water the next day for the eel-like worms. The eggs do occur, but very rarely, and could hardly be distinguished from those of *Uncinaria duodenalis*, but they are perhaps a little larger, measuring from 65 to 70 microns long and from 34 to 39 microns wide, and are very much segmented. In the intestines all stages of the development of the embryo may be followed.

The adult female resembles a filaria; it measures from 2.1 to 2.2 mm. long and 32 to 39 microns wide. The body increases slightly and gradually in size from the head to the posterior quarter, and then terminates rather suddenly in a short tail. The male is about one-fifth smaller.

The worms are abundant in the duodenum, fewer in the jejunum. The adults are found very rarely in the stools. They occur in about 0.6 per cent. of a series of patients examined in Washington.

<sup>19</sup> Deutsch. med. Wochenschr., 1902, June 26.

Houghton, writing from Wuhu, China, states that from 0.9 to 1 per cent. of all patients examined there were infected with this parasite, but, emphasizing the local character of the distribution of parasites, says that this worm has not yet been reported north of the Yangtse River or in Manchuria or Korea.

**Trematodes.**—Infections by *SCHISTOSOMUM JAPONICUM* have recently been carefully studied in the Far East by Katsurada in Japan, by Catto in Singapore, by Woolley in the Philippine Islands, and by Houghton\* in China (in the provinces of Hunan, Honan, Hupeh, Kiangsi and Anhui).

Houghton found that 8 per cent. of all male patients admitted to the Wuhu General Hospital, Anhui, during one year were infected. Almost all of these infected patients were farmers and boatmen from the southern half of the province of Anhui and within a radius of 100 miles of Wuhu. As illustrating the circumscription of the areas of trematode infection, Houghton writes (personal communication) that in the province adjoining Anhui on the north the worm has not yet been found. Houghton states that "the distribution of the parasite in China follows in general the flat, low-lying lands of the central valley of the Yangtse and the valleys of tributary waters; in Anhui at least its presence is limited to the rice-growing divisions of the country. No cases from the hill or mountain districts have appeared." Peake, however,† reports that it is common among raftsmen in Hunan. In some places it is claimed that one in every three or four (some say even more) of the farmers and boatmen shows the physical signs of this infection.

Well-marked cases of this infection have enlarged liver and spleen, cachexia, eosinophilia, ascites, greatly exaggerated knee-jerks, and bloody stools. The eosinophile cells form from 10 to 51 per cent. (average 25 per cent.) of the total count. Less well-marked cases may show only the enlarged spleen and the eosinophilia or the eosinophilia alone. Very few have only ova in the stools. The leucocyte count is not increased, but varies from 2000 to 8500 per cubic millimetre. There is no marked anæmia (Hb averages 80 per cent.).

The ova may be found in the blood, but they appear especially in the stools, although it is not always easy to find them. In size they closely resemble the ova of *Ascaris lumbricoides*, for which, under the low power, they may easily be mistaken; or, rather, an ascaris egg with its mammillary envelope not deeply bile-stained and its bosses not very prominent might easily be mistaken under the low power for an ovum of *Schistosomum japonicum*. The latter ova

\*Trans. of the Society of Trop Med. and Hygiene, June, 1910, vol. iii, No. 7, p. 342.

† China Medical Journal, 1908.

are much more refractile and, since their envelopes are sticky, gather débris in the stool and leucocytes in the blood. (The eggs are of a yellowish-brown color, are oval, have neither operculum nor spine; and measure from 60 to 90 microns in length by 30 to 50 microns in breadth.) In the fresh stool the embryo in the egg is quiescent and shaped like a melon seed; later there is motion of the cilia. The free-swimming miracidium is seen only after the stool has stood about ten hours. It can be kept alive in water for at least five days.

The adult male measures about 10 mm. in length and 0.5 mm. in breadth. The slender, almost cylindrical female is 8 to 12 mm. long and measures 0.113 mm. in diameter. The skin of the worm, unlike that of *Schistosomum hæmatobium*, is smooth. The adult worms are found in the smaller mesenteric blood vessels (perhaps in the arteries especially). The ova are found in necrotic areas in the mucosa and submucosa of both the small and the large bowel, but some are found in the subperitoneal tissue.

*FASCIOLOPSIS BUSKI* (*DISTOMUM BUSKI*, *D. CRASSUM*), the largest of the trematode parasites of man, measures from 34 to 70 mm. in length and from 5.5 to 14 mm. in width. Its eggs are from 120 to 130 microns long and from 77 to 80 microns wide. They have a thin shell, a very small operculum, and granular contents. Only a few cases, all of intestinal infection, have been reported, and these were in the Far East. The infected cases have shown a moderate diarrhoea continuing for years, emaciation, and anæmia. The one parasite described under the name *Distomum rathouisi* was, according to those who have had a good chance to decide, probably a specimen of *Fasciolopsis buski*.\*

*DISTOMUM LANCEOLATUM*.—The body, pointed at both ends, is from 8 to 10 mm. long and from 1.5 to 2.5 mm. wide. The eggs, which are yellowish when young, dark brown when older, have thick shells and measure from 38 to 45 microns in length and from 22 to 30 microns in width. They contain an oval miracidium, of which the anterior part alone is ciliated, and which hatches only in the intestine of some intermediary host, perhaps of a slug. This lancet fluke is a relatively rare parasite of the biliary ducts of the European and American domestic animals. Thus far it has been found but seven times in man.

*FASCIOLA HEPATICA*.—The liver fluke is a widely-spread parasite inhabiting the bile-ducts of many herbivorous mammals. The adult measures from 20 to 30 mm. in length and from 8 to 13 mm. in breadth and has a definite head cone. The ova are yellowish-brown, oval, and from 130 to 145 microns long and from 70 to 90 microns wide, and they have a cap-like lid. The elongated miracidium, which

\* Jeffreys and Maxwell, *Diseases of China*, 1910.

is completely ciliated, escapes from the egg, after this has been in the water a few weeks, and swims free until it enters a water-snail, in which it passes through the stages of sporocyst, redia, and cercaria. The cercaria become encysted on the grass of the meadows and are eaten by sheep, cattle, etc. Only twenty-three cases have been reported in man.

**Cestodes.**—In a suspected case of tape-worm it is always important that segments be seen before the treatment, which is severe, is undertaken. We have had sent to this clinical laboratory, for instance, mucous casts of the intestine under the supposition that they were decomposing tape-worms. Certain food constituents are also thus interpreted. To determine the success of treatment the head should be searched for; the stool is well mixed with water and allowed to settle for ten minutes, and then the upper fluid decanted; this is repeated several times; the heavy head will settle to the bottom. If the head is not found a cure is not certain till three months have passed without the reappearance of segments.

**TÆNIA SOLIUM.**—The infection is derived from *Cysticercus cellulosæ* of pork. The adult worms in the intestine average about 3 m. long, although much longer have been described; the head varies from 0.6 to 1 mm. in diameter, with four suckers from 0.4 to 0.5 mm. in diameter, and a rostellum with a double crown of 22 to 32 hooks from 0.11 to 0.18 mm. long. The neck is about 3 cm. long, and is unsegmented. The ripe segments are from 9 to 10 mm. long, by from 4 to 5 mm. wide. The genital openings are at the margin and arranged in a fairly

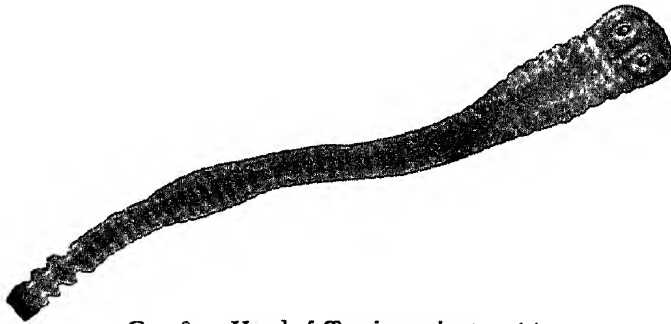


FIG. 87.—Head of *Tænia saginata*.  $\times 5$ .

regular alternating manner. The uterus is characteristic, consisting of a large median stem and on each side from seven to ten coarse branches, each one of which branches dendritically. The eggs are round or oval, the shell very thin but surrounded by an embryonic shell which is thick, with radiating lines, and often yellow in color. These eggs are about 35 microns in diameter. This worm is excessively rare in America. The only specimen of *Tænia solium* which I have seen was discovered in Baltimore by Dr. Thos. Boggs. Those exhibited in museums are mostly wrongly labelled (Figs. 86 and 89 A).

**TÆNIA SAGINATA.**—The beef tape-worm, the infection arising from the cysticercus of beef and, perhaps, of sheep, is in this country quite common. The adult worm varies from 4 to 8 m. or more in length. The head (Fig 87) is from 1.5 to 2 mm. in diameter,

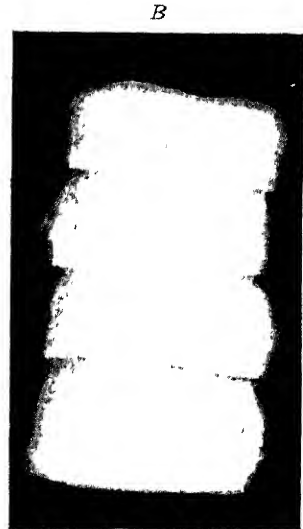
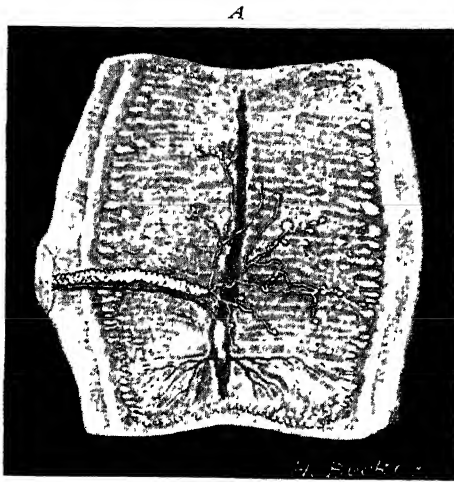


FIG. 88 —A, ripe link of *Tænia saginata*.  $\times 3$ . B, four unripe links.  $\times 3$



FIG. 89.—Eggs of *Tænia saginata*.  $\times 400$ .

A. H. Mors† Rec



Nat size

$\times 4$



FIG. 89a.—*Tænia solium*. Mature links from a case recently discovered in the Johns Hopkins Out-patient Department. (Kindness of Dr. T. R. Boggs.)





cuboid in shape, with four suckers each 0.8 mm. in diameter, and without hooks. The neck is long and delicate. The ripe segments (Fig. 88) are from 16 to 20 mm. long by from 5 to 7 mm. wide. The over-ripe segments are longer and somewhat more slender. The genital openings are at the margins and irregularly alternate. The uterus is characterized by the multitude of its fine branches, from twenty to thirty-five on each side of the median stem, which branch dichotomously. The eggs (Fig. 89) are spherical, with a thin shell surrounded by the embryonic shell which is thick and radially striated,

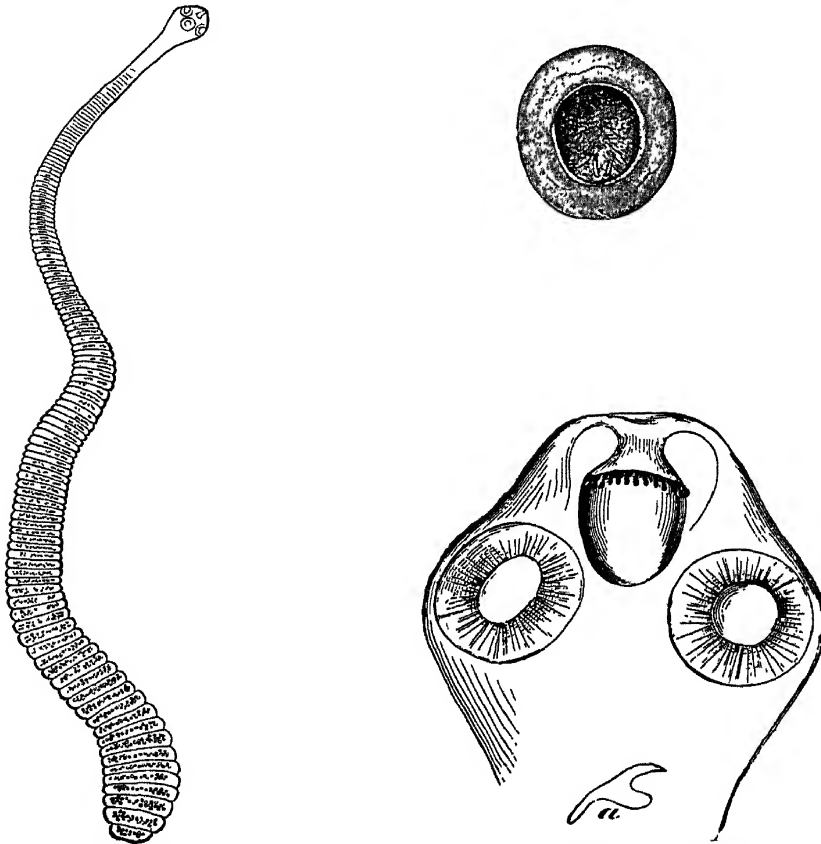


FIG. 90.—*Hymenolepis nana*. Adult (left), head (right), egg (above); a, hooklet. (From Braun.)

transparent, oval, from 30 to 40 microns long by from 20 to 30 microns wide.

*HYMENOLEPIS NANA*. *TÆNIA NANA*.—This is a dwarf tape-worm not at all uncommon in man, as Stiles<sup>20</sup> has shown who found it in sixteen of three thousand five hundred persons.

The worm (Fig. 90) is from 10 to 15 mm. long, from 0.5 to 0.7 mm. broad, with four suckers and a row of twenty-four to thirty very small and characteristically shaped hooks (14 to 18 mi-

<sup>20</sup> New York Med. Journ., 1903, vol. lxxviii. p. 877.

crons long) on the spherical head, which is 0.25 to 0.3 mm. in diameter. The segments, about one hundred and fifty in number, are short (14 to 30 microns) and relatively broad (0.4 to 0.9 mm.).

The egg is characteristic; it is spherical or oval, 30 to 37 by 48 microns in diameter, having two distinct thick membranes, each pole of the inner having a more or less conspicuous process with filamentous appendages.

The parasite inhabits the ileum, where a few or many thousands may be present. It is probably the same as the very common form in rats.

**DIPYLIDIUM CANINUM. TÆNIA CUCUMERINA.**—This tape-worm is from 15 to 35 cm. long, from 1.5 to 3 mm. broad, the head club-shaped with rostellum and three or four rings of hooklets. The eggs are circular, from 43 to 50 microns in diameter, with thin shell. It occurs in dogs, cats, and rarely in man, and then especially children.

**BOTHRIOCEPHALUS LATUS.**—This tape-worm (Fig. 91), the largest parasite of man, is exceedingly common in the maritime countries of Europe, in Ireland, and in Japan.

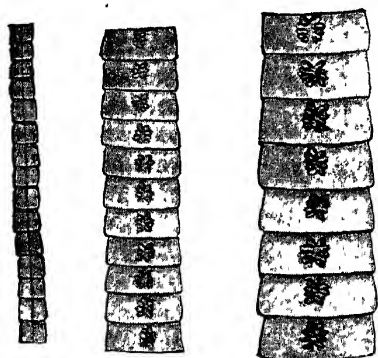


FIG. 91.—*Bothriocephalus latus*.  
(From Braun)

The cysticercus stage occurs in fish. A rapidly increasing number of cases is being discovered in America. A very good report is that of Willson.<sup>21</sup> This tape-worm is often 8 m. in length and in some cases has reached even fifty feet. The infections are often multiple. In Willson's case there were two worms with an aggregate of eighty-two feet. In the multiple infections with fifty to one hundred worms the individuals are much shorter, sometimes from three to five feet long. The

head is 1 mm. broad, from 2 to 3 mm. long, is flat, almond- or spoon-shaped, with two deep grooves at its sides which serve as suckers. It has very little neck. The ripe segments, which begin about 50 cc. from the head, increase in size until about 10 to 15 mm. broad and 3 to 4 mm. long. The genital opening is on the side, not the edge, and around it the uterus is arranged as a rosette. The distribution of these organs is more regular than that of the septa of the segments, Willson considering the imperfect or abortive segments very characteristic of this family of worms. The eggs are characteristic. They have a thin shell, the contents coarsely granular, mulberry-like, and a lid which may be open or closed. In very young eggs the lid cannot be seen, and in older may be rendered evident by pressure

<sup>21</sup> Amer. Jour. of Med. Sci., 1902, vol. cxxiv.

on the glass. Their size is 70 by 45 microns. The eggs are important in diagnosis, since the segments occur only at certain times, although when they do occur it is in abundance. The most interesting thing about this enormous tape-worm is the production in certain of the hosts of an anæmia which hæmatologically cannot be distinguished from primary pernicious anæmia. Only a small percentage of cases which harbor the worm have this anæmia, and they recover rapidly when the worm is removed. Various reasons are given for the good health some hosts enjoy,—heredity, lack of predisposing causes, etc., while Dehio thinks the worm to be harmful must either die or at least be diseased. The cause of the anæmia is pretty certainly a toxine affecting the blood and perhaps the bone marrow.

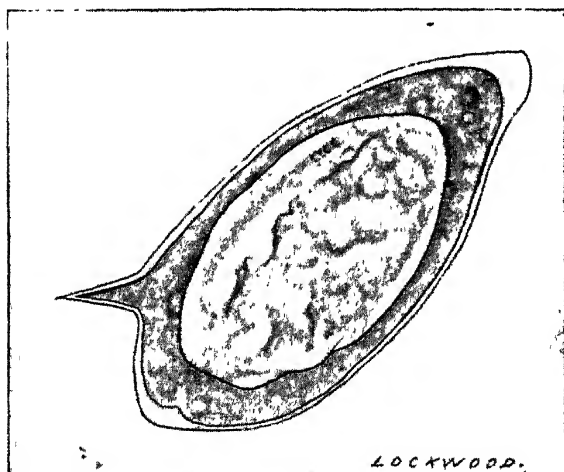


FIG. 92a.—Egg of *Schistosoma hæmatobium* found in stool. Embryo dead.  $\times 400$ .

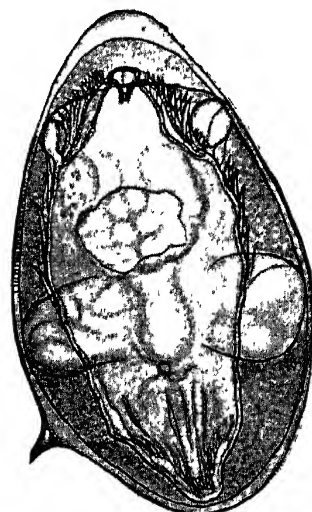


FIG. 92b.—Egg of *Schistosoma hæmatobium* found in stool. Embryo alive.  $\times 400$ .

The eggs of *SCHISTOSOMA HÆMATOBIUM* (see page 311) may also be found in the stools. It is interesting to note the number with the spine lateral and those with it terminal.

**Plant Parasites.**—YEASTS are often present in normal stools. MOULDS are rare. Blastomycetes were found in the stools of patients with systemic infection with this parasite.\* The *OIDIUM ALBICANS* has very rarely been found in children. *SARCINÆ* are often found in cases of dilated stomach. When present in large numbers they may aggravate a diarrhoea by the products of their fermenting processes.

**BACTERIA.**—Bacteria form no small part of the mass of the stools (see page 401), the vast majority of these organisms are dead. Almost any organism may appear accidentally in the intestine, but there is a flora of bacteria so constantly found that their presence may be considered normal. Among these are: *Bacillus coli communis*

\* Fontaine, Haase, and Mitchell, Arch. Int. Med., Aug., 1909.

(see page 294), *Bacillus lactis aërogenes* (see page 295), and for the suckling, *Bacillus bifidus*.

**BACILLUS BIFIDUS** (Teissier, *Paris Thesis*, 1900).—This organism (see Fig. 92c) would appear to be a normal inhabitant of the intestines of the suckling, and to disappear soon after the child is weaned. When, however, it persists, its presence would seem to be associated with certain symptoms of intestinal intoxication. It is an organism from 2 to 4  $\mu$  long, and often in pairs. Its most characteristic shape is like the letter Y. Involution forms are common. The greatest interest to us is that this is one of the few intestinal organisms which is not decolorized by Gram's method. It is a strict anaërobe. Among other important organisms are, *Bacillus alkaligenes* (see page 296), and the proteus group (see page 296). Among the important patho-



FIG. 92c.—*Bacillus bifidus*. (Photomicrograph by Dr. Thomas M. Wright.)

genic organisms which sometimes, even frequently, are found, are *Bacillus pyocyaneus* (see page 296), *Bacillus aërogenes capsulatus* (see page 296), *Bacillus tetani* (see page 297), the *Staphylococcus* group (see page 297), and the *Streptococcus* group (see page 298). A great many of the *thermophilic* and the *acidophilic* organisms are present, and they were recently described as the predominating organisms among those which were supposed to be dead since they would not grow on ordinary media, or at ordinary temperature. (The thermophilic organisms grow only at temperatures above 40° C. and some of them best at 60° C. They cannot therefore multiply in the intestine and those found in the stools must be swallowed with the food.) The present opinion is that the lower bowel at least is not a favorable habitat for organisms and that the most in the stools are dead.

**TUBERCLE BACILLI.**—In a search for these it is useless to digest a solid stool. Mucous masses should be selected, especially the blood-

stained or purulent particles, and these treated as in sputum. In class work it is interesting to note how many some find who select the particles with care, and how many students find none. In intestinal tuberculosis they are often present, and yet in cases in which they would be most expected none are found, hence the probability is that many are destroyed. Again, when found, the possibility of their origin from swallowed sputum must always be thought of, and the diagnosis of pulmonary tuberculosis has been made in this way, especially in children; but this is rather a remote possibility in the case of a careful adult.

Page's method of searching for the bacilli in the solid stool is to suspend a piece half the size of a pea in 1.5 cc. of distilled water, add 54 cc. of a mixture of equal parts of absolute alcohol and ether, and centrifugalize for ten minutes; a smear made of the sediment is fixed to the slide with a drop of egg albumin, and stained as usual.

**Stools in Disease.**—In *typhoid fever* the stool most characteristic is of "pea-soup" appearance, copious, watery, of foul odor, alkaline reaction, with many triple phosphate crystals. But in clinics which limit these patients to a rigid milk diet, diarrhoea is less common than constipation. The stool is frequently blood-tinged, this tingeing sometimes warning us of a coming hemorrhage. Pus (microscopic) is rare except in severe cases with extensive ulceration.

*Bacillus Typhosus.*—Many methods have been proposed for growing this organism from the stools. For a critical review of this subject, see Pratt, Boston M. & S. Jour., 1907, vol. 156.

Drigalski and Conradi's medium.\*

Three pounds of minced beef are mixed with 2 litres of water and allowed to stand over night. The beef is then pressed, the beef juice is boiled for one hour, filtered, and to this filtrate are added 20 gms. of peptone (Witte), 20 gms. of nutrose and 10 gms. of sodium chloride. It is then boiled again for one hour and filtered. To this filtrate are added 60 gms. of the best agar. It is then boiled for three hours (one of which is in the autoclave). The fluid is made faintly alkaline to litmus, filtered, and then boiled for one-half hour. While hot the next solution is added.

The litmus solution is made by boiling 260 cc. of litmus solution for 10 minutes, adding 30 gms. of the purest lactose and boiling again for fifteen minutes. This litmus-lactose solution is now added while boiling to the hot agar solution mentioned above. The mixture is then well shaken and then made very faintly alkaline.

One then adds 4 cc. of a hot sterile 10 per cent. solution of soda and 20 cc. of a freshly prepared 0.1 per cent. solution of Krystallviolett B., Höchst, in warm sterile distilled water.

The resulting medium is a beef juice-nutrose-agar-lactose-litmus solution which contains 0.01 p. M. Krystallviolett. It hardens to a very firm mass. It is firm enough to prevent much diffusion of the acid formed. It does not become dry. The lactose is split by *Bacillus coli*, not by *Bacillus typhosus*. The colonies of colon bacilli will therefore turn red, the typhoid blue (since this organism splits off basic bodies from the proteids). Krystallviolett will inhibit the growth of many

\* Zeitschr. f. Hyg. u. inf. Krankh., 1902, xxxix, 283.

other organisms, especially acid-producing cocci. The medium is poured at once into large Petri's dishes or kept in 200 cc. flasks.

These authors recommend to inoculate several series of plates in order to get the largest possible number of isolated colonies on a plate. If the stool is fluid one series of plates is inoculated with the undiluted stool, another with the stool diluted with 10 to 20 volumes of sterile normal salt solution. If the stool is solid it should be rubbed up to a homogeneous mass with sterile salt solution and various dilutions of this used.

The stool is rubbed on the surface of the medium. After inoculation the plates are left open for at least half an hour to allow the surface to dry, otherwise the colonies will coalesce (the Krystallviolett will kill any air contaminations). When dry the plates are put into a thermostat at 37° C. The plates are examined in from 14 to 24 hours. The colon colonies will vary from 2 to 6 or more millimetres in diameter, they are of a great variety of shades of red in color and are opaque. The paratyphoid colonies are sometimes red, sometimes blue (see page 295).

The typhoid colonies have a diameter of from 1 to 3 mm., are blue or violet in color, glassy, not doubly refractile, seldom opaque.

The colonies of the *Bacillus subtilis* group are also blue, but are so much larger than the typhoid that error is seldom possible. In some fetid stools the blue colonies of *Proteus*, *Bacillus fluorescens* and *Bacillus fœcalis alkaligenes* may deceive. They are rare and can be distinguished by the agglutination test.

Peabody and Pratt\* have shown the value of the following method. Malachite-green-bouillon is first used as an enriching medium. (The beef bouillon they used contained 1:1000 malachite green and had an acidity of 0.5 per cent. to phenolphthalein, but the amount of dye and the acidity must be determined for each preparation of the malachite green used.) While this completely inhibits the growth of *Bacillus coli*, *Bacillus typhosus* will often grow luxuriantly in it, although the dye does exercise some restraint over this organism. Tubes containing from 10 to 15 cc. of this medium are inoculated with one drop of the fluid stool or suspension of the stool and are left in the thermostat from 18 to 24 hours, and then one drop of the culture is rubbed over the surface of a Drigalski-Conradi plate.

Drigalski and Conradi were able to obtain the typhoid bacilli in every case of typhoid fever examined. Pratt, Peabody and Long (*Jour. Am. Med. Assn.*, 1907, xlix, 846) were able to find it in but 21 per cent. of the febrile cases. These bacilli were most numerous in those stools which contained blood. Many think that possibly all

\* Boston Med. and Surg. Jour., Feb. 13, 1908.

the stools of patients with typhoid fever contain a few typhoid bacilli, but that the great majority of the vast numbers which enter the duodenum with the bile are destroyed in the intestine, and only a few are alive when the stool reaches the rectum.

In severe *Asiatic cholera* the rice-water stools are quite characteristic. These are copious, the water being in good part secreted by the intestinal wall, with small gray flecks which are masses of epithelial cells, cholera spirilla, and fat droplets. They have no fecal odor, are alkaline, almost acholic, sometimes are blood-stained, and contain little albumin and much salt.

*Spirillum Cholerae Asiaticæ*.—The spirillum of Asiatic cholera, or the “common bacillus,” is a small curved “comma-shaped” bacillus about  $2\ \mu$  long and  $0.5\ \mu$  thick. It is very actively motile, and has a single long delicate flagellum at one end. It does not produce spores. Involution forms are common. It stains readily in all bacterial stains and decolorizes by Gram’s method. This organism grows very rapidly at room temperature on all ordinary media, and in some so poor in nourishment that other organisms cannot grow in them. It will not grow on potato at room temperature, but will in a thermostat. It is a very aërobic organism. It grows in a fairly characteristic manner on gelatin, which it liquefies. In gelatin plates the colonies soon appear as minute white points which resemble fragments of broken ground glass with granular irregular margins. Then liquefaction begins and the colony sinks in the little cup of liquid cloudy gelatin which forms a halo around it. This organism produces much indol. It is very sensitive to acids.

Other and non-pathogenic spirilla are very common. There is one in the mouth which will not grow in ordinary media. More than sixty non-pathogenic species with similar morphology, but different cultural characteristics, have been found in various drinking waters (*e.g.*, *Spirillum schuylikiliensis*).

Many pathogenic forms have been described: Metchnikoff’s spirillum was found in an epidemic in chickens, is pathogenic for birds (the true cholera spirillum is not), and is a more rapid grower than the latter; Masea’s spirillum (very pathogenic to pigeons and with four or five flagella); Finkler and Prior’s form, from a case of cholera nostras (grows as a dirty brown scum on potato at room temperature); Deneke’s form from old cheese (will not grow on potato).

But morphology and cultural characteristics are not sufficient for the recognition of these organisms. The specific test must also be positive. A guinea pig is immunized to a given species of the spirillum. A little of the organism in question is then injected into the peritoneal cavity. If the organism injected is the one to which the animal is immune, those in the peritoneal cavity will be rapidly destroyed.

The diagnosis of Asiatic cholera may (25 to 30 per cent. of the cases in some epidemics) be made directly from the stools. A smear will show vast numbers of these comma-bacilli.

Usually it is necessary to inoculate gelatin and agar plates with "rice particles" of the stool and in 24 hours (if grown on gelatin at 22° C.) the typical colonies are found. When there are but few organisms present the enriching method of Schottelius is used. If a little of the stool is well mixed in a large amount of bouillon, a surface scum of these spirilla will form, from which cultures may be made.

To demonstrate this or similar organisms in water, add to 90 cc. of the suspected water 10 cc. of a sterile solution of 10 per cent. peptone and 5 per cent. of sodium chloride. This is now placed in a thermostat and the scum examined later.

IN DYSENTERY, RECTAL DIARRHŒA, and CANCER OF THE RECTUM, movements are frequent and scanty. They soon lose their fecal character and become mucoid, mucopurulent, hemorrhagic or sero-hemorrhagic, the amount of blood separating the cases of "white" from "red" diarrhœa. Sometimes fragments of necrotic mucous membrane or of cancer are found; often masses of bloody mucus.

IN AMŒBIC DYSENTERY during the acute exacerbations there is diarrhœa of from three to six movements a day containing bloody mucus in which may be found the amœbæ; the stools are thin and watery, their odor offensive. These periods are separated by others of even years' duration, with normal movements, or constipation, and yet here the amœbæ may be found if looked for. It is in these cases that the routine examination of the stool is important, even in cases without symptoms referable to the liver, as was seen in a recent case of constipation and irregular fever without hepatic or intestinal features. At autopsy a large amœbic abscess of the liver was found.<sup>22</sup>

THE DYSENTERY BACILLI.—In shape and in some of its cultural characteristics "Shiga's Bacillus" resembles *Bacillus typhosus*. It is a short organism with rounded ends and is inclined to involution forms. All now agree that it is non-motile. No spores are formed. This organism stains readily in the commonly used aniline dyes, showing a tendency to polar staining, and is decolorized by Gram's method.

Since Shiga's discovery twelve organisms of dysentery have been described, all belonging to one group, all with similar morphology and similar staining characteristics, all non-motile, all unable to liquefy gelatin, to form acid from lactose, and to form gas from any carbohydrate. They differ in the amount they agglutinate in immune sera and in their ability to ferment carbohydrates. His recognizes:

<sup>22</sup> See also Councilman and Laffeur, *Johns Hopkins Hosp. Rep.*, vol. ii, p. 395.



Group I.—“Shiga,” “Kruse,” and “New Haven.” These ferment dextrose.

Group II.—“Y” (His and Russell type), “Seal Harbor,” “Diamond,” and “Ferra.” These ferment dextrose and mannite.

Group III.—“Strong” (type). This ferments dextrose, mannite and saccharose.

Group IV.—“Harris” (type), “Gray,” “Baltimore,” and “Wollstein.” These ferment dextrose, mannite, saccharose, maltose, and dextrin.

Flexner recognizes three types:

1. The “Shiga type,” which ferments glucose only.
2. The “Flexner-Harris type,” which ferments glucose, mannite, and dextrine, but not lactose. This is the type which prevails in the United States.
3. *Bacillus* “Y” (His and Russell), which ferments only glucose and mannite.

These organisms are the causes of “bacillary” or “infectious” dysentery, which may occur sporadically or in epidemics, *e.g.*, the severe epidemics of tropical dysentery. The disease begins as an acute gastro-enteritis with a diarrhœa which increases in severity until the stools lose their fecal character, are very frequent and scanty, and contain chiefly mucus and blood and numerous organisms of dysentery.

In recognizing these organisms the agglutination tests are most important. The blood serum of a patient infected with an organism belonging to the Flexner-Harris type will agglutinate the pure culture of this organism in dilutions of 1:1000–1500. In the case of the Shiga bacillus agglutination is less complete.

*Pancreatic Disease.*—Fatty stools are common (see page 406), yet this must be confirmed by other signs of pancreatic disease. A large amount of muscle-fibre (Azotorrhœa) in a case without diarrhœa is a valuable sign; a reduction of ethereal sulphates in the urine is a point in favor, yet alone is of little value; yet all together are of value in a case without jaundice and with other signs of pancreatic disease, as abdominal tumor, glycosuria, etc. In some cases of diabetes the stool is pure fat, in amount from a few drachms to a cupful of pure yellowish-brown fat; in others about 30 per cent. is fat. Naunyn knows of no case of true fatty stools in diabetes without pancreatic disease.

Atkinson and Hirsh\* reported one of the most typical cases of severe chronic interstitial pancreatitis due to pancreatic lithiasis. The patient, who had diabetes mellitus, on an unrestricted diet evacuated four litres of fæces daily. The stools, soft or semi-solid, and leathery-

\* *Am. Jour. Med. Sci.*, Oct., 1907.

brown in color, had a penetrating odor like rancid butter and contained 54.6 per cent. of fat (22.5 per cent. neutral fat and 32.1 fatty acid), which was present in microscopic masses and in macroscopic lumps varying in size from that of a split pea to that of a walnut.

For Sahli's test of the efficiency of pancreatic juice, see page 401.

A recent method highly recommended is the use of pancreon, which preparation is not affected by the gastric juice. In a case with much muscle in the stool and no diarrhœa, if pancreon be given and the muscle diminish, it is in favor of pancreatic disease.

Some (*e.g.*, Ury and Alexander)<sup>23</sup> would base the diagnosis on fatty stools, many muscle-fibres in a fairly solid stool, and failure of jaundice. The suspicion is especially justified if a large amount of fluid fat separates itself from the rest of the stool. The simultaneous presence of glycosuria is rare, the absence of decomposition is not usual, nor should it be expected since there is so much albumin present, but the simultaneous steatorrhœa and azotorrhœa is important and with diabetes conclusive.

And yet in pancreatic disease azotorrhœa may wholly fail. Again, steatorrhœa alone is not very important, for with complete atrophy of the pancreas this may persistently fail, and if present it may be due to a long list of diseases affecting fat absorption as well as fat splitting. And in some cases with increased fat the per cent. split is normal. All will agree that stool examination is of little value unless the patient has been on a constant diet of known composition. As yet not enough work has been done to detect more than gross variations. The assimilation limit for fat in a normal case is about 350 gms. of butter; of this the loss is not over 7 to 10 per cent.; jaundice or acholia due to other diseases must not be present; the fat should not be emulsified; any diarrhœa should be checked with opium when making such alimentary tests.

PERMANENT MOUNTS OF SMALL WORMS.—For the following methods I am indebted to Dr. Thomas R. Boggs.

*Boggs' Method.*—The worm is allowed to die in water (that it may be fixed while in a relaxed condition). It is then spread out on a piece of filter paper and immersed in an alcohol-glycerin cleaning fluid (alcohol, 80 per cent., 16 parts, and glycerin 4 parts). The specimen is allowed to remain in this solution in an open dish loosely covered with cloth or paper, until the alcohol has entirely evaporated off. This may take from two to six weeks. Since the worm is spread out on the filter paper it will contract but little. Should it do so it may be slightly pressed between slides held together by rubber bands. When the specimen is sufficiently clear it is gently blotted on the

<sup>23</sup> Deutsch. med. Wochenschr., 1904, No. 36.

slide and covered with melted glycerin jelly. The cover-slip is then dropped on and if necessary pressed down until the jelly has hardened. After the jelly is hard the excess is removed from the borders of the cover and the edges sealed with microscopic cement or asphalt paint.

The glycerin jelly is made by melting 14 grammes of the best gold mark gelatin in 120 cc. of hot water, and adding 120 cc. of glycerin. This is then cooled to 50° C. The carefully separated whites of two eggs are then added and the fluid heated gently without stirring. This is then filtered, the volume made up by adding water to 240 cc., and 1 cc. of pure carbolic acid is added. This jelly is solid at ordinary temperatures, but is easily melted under the hot water tap.

TO PRESERVE STOOLS CONTAINING PARASITE EGGS.—The stools are diluted to a soup-like consistency and then one-tenth volume of formalin is added. The specimens are then kept in a tightly corked bottle. Parasite eggs and larvæ will keep fairly well preserved.

#### FLAT WORMS.

*Preservation of the Gross Specimen.\**—The best way of getting the worms in a clean condition is by mixing the fecal matter with an amount of warm (37°–40°) normal salt solution sufficient to make a thin broth. If the specimens are obtained at autopsy, the intestinal contents may be washed or scraped off into the salt solution, in which the worms move about freely and are easily seen and isolated, especially small worms, such as *Hymenolepis nana*. With a pair of finely-pointed forceps the parasites are picked up and transferred to a second dish of warm solution, where they will be found to be clean. Specimens for sections and for mounting should be taken from the solution and treated with the proper fixatives. The rest of the material may be placed in 50 or 70 per cent. alcohol with or without glycerin, or in Zenker's solution, or in a 2 per cent. solution of formalin. Zenker's solution causes considerable shrinking and a rather marked yellowish discoloration. These authors consider the formalin mixture much better, as it preserves the natural whiteness and causes little or no shrinkage.

*Preparation of Segments for Mounting.\**—The specimens are washed in normal salt solution (0.85 per cent.) and fixed by keeping them in 2 per cent. formalin from fourteen to sixteen hours. They are then transferred to the following glucose medium, which is a slight modification of the Fabre-Domerque medium:

Sirup (glucose, 48 parts; water, 52 parts).....	1,000 cc.
Methyl alcohol ....	200 cc.
Glycerin .....	100 cc.
Camphor ( <i>q.s.</i> , to keep).....	100 cc.

\* Mink and Ebeling, U. S. Naval Med. Bull., No. 3, vol. iii.

In this medium the specimens may be left indefinitely, although it has been found that they clear sufficiently within four or five hours. In mounting them, a sufficient quantity of Keisser's glycerin jelly is dropped on a slide, and the specimen is transferred to this, care being taken not to admit air-bubbles. A cover-glass which has been passed through the flame finishes the mount. After the glycerin jelly hardens a few coats of gold-size applied around the cover-glass furnish rigidity and improve the general appearance of the preparation. Concave slides are desirable when the specimen is of uneven thickness or rather thick throughout.

*Preparation and Sectioning of Material.\**—To prevent curling or distortion of the worm or segment, a fixative is used which kills quickly, and for this Zenker's fluid is best. About 3 or 4 inches of the live tape-worm are taken from the salt solution and stretched out on an ordinary glass slide.

By means of a pipette the slide is rapidly covered with Zenker's fluid. The section of the worm straightens out, hardens, and floats on the solution. It may then be transferred to a flat dish filled with Zenker's fluid and remain there from two to twenty-four hours. By cutting slightly beyond the part needed for work one leaves small end pieces which may be grasped with the forceps in subsequent manipulations. Thus the segments used for study need not be touched with the forceps. The later processes include treatment in an alcohol-iodine solution, in graded alcohols for dehydrating, and other steps until the tissue is immersed in melted 45° C. paraffin. In blocking the specimen it seems best to place the longest and broadest surface downward and later trim and mount it as desired. Specimens are best cut either in planes parallel with the long, broad surface, or perpendicular to the long axis. Sections are cut from 25 to 30 microns thick. The most convenient stain is a rapidly acting, purely nuclear hæmatoxylin.

**STAINED SPECIMENS OF WORMS.**—The worm, as fresh as possible, is fixed in a boiling saturated alcoholic solution of mercuric chloride for from 10 to 30 minutes, depending on the thickness of the specimen. It is then washed in running water over night, and then in water containing a trace of tincture of iodine until it is free from mercury. The specimen is then heavily overstrained (for from 12 to 24 hours) with hæmatoxylin or carmine, and decolorized with acid alcohol, under the lower power of the microscope, until the desired color is obtained. The specimen is then washed, dehydrated in alcohol, cleared in oil of cloves or creosote, and mounted in Canada balsam. This method is best for the study of the minute anatomy of tape worms and flukes. It is not so successful with round worms.

\* Mink and Ebeling, U. S. Naval Med. Bull., No. 3, vol. iii.

## CHAPTER V

### THE BLOOD

**Obtaining the Blood.**—A simple sharp-pointed lancet is necessary, or a needle with a cutting edge. Of the latter there are on the market several with a bayonet point. A good instrument is the ordinary Hagedorn needle, which the surgeons prefer for blood work. If nothing better is at hand, an ordinary steel pen, one nib of which is broken off, will do excellently. The one thing to remember is that the point should have a cutting edge, and not be round and sharp, not too long and slender, as is so often the case. There are on the market also holders in which separate needles can be inserted. These can be thrown away when dull, thus obviating the necessity of sharpening the instrument. Special forms have been invented, some with the needle on a spring which forces it to a certain depth, as, for instance, Francke's needle, which is quick and painless, and is to be recommended to those who cannot train themselves to give a sharp, quick blow. In another form the needle projects from a holder which serves as a guard, and hence, no matter how hard a blow is given, can penetrate only a certain distance. An illustration of this is the Daland needle. We prefer, however, a simple instrument, any needle with a cutting edge; and if the hand be at all trained, a puncture can be made deep enough to get as much blood as is necessary.

If considerable blood is desired, a hypodermic syringe should be used. A tight bandage is tied around the upper arm, and then a towel wet with warm bichloride wrapped around the elbow-joint, which has been previously cleaned up. The needle is inserted into the distended median basilic vein at the end of the elbow. For quantitative blood work, however, after the needle is inserted the bandage must be removed and circulation be allowed to return to normal before any blood is withdrawn, since the stasis has altered the concentration of the blood.

Two forms of forceps are necessary (Fig. 98), the one with the crossed points which will hold one cover-glass, and the other the ordinary straight pinch forceps, with which the second cover-glass is to be handled. In the case of the first-mentioned forceps the two arms should come in contact for the whole length of the blade, and therefore hold one edge of the cover-glass solidly. The spring of the second pair should be as weak as possible, since when large numbers of cover-glasses are to be handled a stiff pair will weary the hand considerably. Beginners prefer to handle one cover-glass with their fingers, and

although this is advised by many good authorities, we insist that it shall not be done in this clinic, because the technique is certainly better if the fingers do not touch the cover-glasses. Also after the worker has become accustomed to the forceps, he can work more rapidly and accurately with the two than if he uses his fingers for one.

The glass slides must be thoroughly cleaned for use in blood work. They have often a right and a wrong side, since they are cut from a flattened cylinder of glass; in some boxes almost all are slightly concave on one side and convex on the other, since the flattening was not perfect. If the blood specimen is on the concave side the slide will rock on the stage of the microscope, hence making it impossible to keep a field in focus, while if the specimen is put on the convex side the slide will rest firmly on its two ends.

The cover-glasses should be thin, No. 1, or preferably No. 0, and three-quarters of an inch square. Cover-glasses seven-eighths of an inch square are a little too large for blood work. In general only new cover-glasses shall be used. The reason for this is learned by bitter experience, because it is almost impossible to clean up a cover-glass so that small masses of detritus or hæmoglobin will not remain on the glass, and the student will think it pigment or some other unusual thing in his next blood specimen.

To clean this glassware considerable care is necessary. The technique will depend on the state in which they are found when bought. We have received boxes of cover-glasses and slides which it was so difficult to clean that we discarded them. As a rule, however, there is little difficulty, and to wash them off in soap and water, then clean water, then in 95 per cent. alcohol is sufficient. If necessary, they should be soaked about twenty-four hours in concentrated hydrochloric acid, then washed in water, then in 95 per cent. alcohol, then in ether. It is well to keep them either in 95 per cent. alcohol or, carefully wiped, in a glass dish. They should be handled only with forceps. For wiping them an old linen handkerchief is, we think, by far the best, since the repeated ironing has removed the most of the lint. A blood-worker should always have at hand a plentiful supply of clean glassware.

In obtaining the drop of blood considerable care should be taken to select that portion of the body which promises the best results. To always prick the ear or always one finger is a decidedly foolish habit, since in some cases the one will serve much better than any other. A general rule, however, should always be observed,—to avoid any part which is cyanosed or œdematous. We have seen counts made on the two ears at the same time in which the leucocyte count varied by 100 per cent., and the same may be said of the two hands. The ear is usually the best part to prick, since it is always within reach,

a nervous person cannot watch the worker, and it is relatively painless. In young children this last point is particularly true. And yet, if the lobe of the ear be small and thin it will be difficult to get a drop of blood from it. If the lobe of the ear is thick it is usually pricked on the flat side, it being stretched over the index-finger by the thumb and middle finger. In case, however, it is thin and considerable blood is necessary, it is well to prick on the edge and parallel to the surface. In cases of pernicious anæmia the ear usually offers much the best opportunity to get a good drop of blood. The Germans, as a rule, take the palmar surface of the ball of a finger of the left hand, and can usually get a good drop of blood. Our students, in studying their own blood, have learned to search on the anterior surface of the forearm for the pain points, and avoiding these, to always prick over a small superficial vein. In this way a good drop of blood is obtained easily and painlessly. In the case of very small children, the great toe or the heel is to be preferred. Each individual case should be studied, and before the prick is made the observer should decide what part of the body offers the best opportunities, whether the ear or the finger, the forearm or the foot, always remembering, however, that a congested or a cyanosed or an œdematous part is to be carefully avoided. It is particularly desirable, in case the patient is to be pricked at least twice a day, true of our pneumonia cases, to vary the parts selected so that they will not get sore. The patients will very soon tell us where they wish the drop of blood obtained. The needle need not be sterilized, but is first dipped into alcohol. The skin is washed off with alcohol and perfectly dried.

The method of "stabbing" varies. Some prefer a short, quick, sharp blow; others, slow pressure. Which method is to be recommended depends on the person using it. In general it may be said that in case considerable blood is desired a slow steady deep prick is better than a quick one. The patients much prefer a prick that is too hard rather than several which are not deep enough, and the unfortunate accident of going through the entire lobe is for the patient not so uncomfortable as the several light blows which beginners are apt to give. The part pricked should not be squeezed, nor should it be held in a position which will check its circulation, nor should it have been rubbed to increase circulation at that point, nor should by hard pressure the flow be aided, since by all of these methods the concentration of the blood may be slightly changed. The drops should well out. The first is wiped off, the second used. To encourage the flow a slight pressure probably does no harm, but to squeeze is not good technique. In case many drops are to be taken, the incision should be wiped occasionally with an alcohol sponge and then with a dry sponge, since this will keep the place bleeding better than a dry

sponge alone. Always ask for a history of hæmophilia, for the bleeding from even a small prick is in those cases difficult to check, and the very slightest prick will furnish enough or even too much blood.

**The Examination of the Fresh Blood.**—The examination of the specimen of fresh blood is very important, and should be a routine procedure in every possible case. Yet many neglect it for the study of stained specimens, which should be considered of secondary value. In the majority of cases, of course it is the stained specimens which we study, since it is not always or often convenient for the practitioner to study fresh blood. We speak now of the relative value of the two examinations, other things being equal. Sometimes information of the highest value and of a very unexpected nature is learned from a fresh preparation; more often hints are gained suggesting to the worker along what further lines to work. He will thus make examinations of which he might not have thought, and can save himself the time of some done as a matter of routine. Some things can be studied only in the fresh specimens, and more can be better studied there than in the stained.

**Technique.**—The slide and cover-glass must be perfectly clean (see page 448). It is well that the slide be slightly warmed, perhaps by rubbing it rapidly with a cloth or holding it an instant near a flame, since the blood spreads much better on glass at about body temperature than it does on a cold one. The size of the drop is a matter of considerable moment. One about as large as the head of the common small black-headed pin is right in most cases. The skin is punctured, the first drop wiped off, and the second or a later picked up from the ear by means of a cover-glass held in the pinch forceps, care being taken that the cover-glass does not itself touch the skin, and is then dropped at once onto a slide. The blood should spread evenly. Under no condition should the cover-glass be pressed down or tapped with the forceps to aid spreading, since this does not make a poor specimen good, and does too great mechanical injury to the corpuscles. After the drop has once touched the slide it is needless to say that the cover-glass, even though it projects over the edge, cannot be pushed into a better position. The student should always make sure that he puts the cover-glass on the convex side of the slide, since it is exasperating to have a rocking specimen under the microscope. The drop should be so small that when well spread the blood film hardly reaches the edge of the cover. The reason for this is that the observer needs the whole of a specimen, however small, under observation; and since the distribution of cells varies at different parts of the slide, there is an advantage in a small drop.

**Red Blood-Cells.**—In the well-made specimen the red corpuscles will all lie singly, flat on their sides, not overlapping nor in rouleaux. But



in some cases it is important to know whether the tendency to rouleaux formation is increased or diminished, since in some diseases there is none, while in others it is so much increased that all the red blood-corpuscles are badly clumped. To test this a large drop of blood is used.

The NUMBER of the red blood-cells may be guessed at with a certain degree of accuracy by one who always uses approximately the same sized drop of blood.

The SHAPE of the red blood-cells is of considerable moment. In the circulation they may be cup-shaped, but in a well made specimen they flatten out on the glass, showing a biconcavity. When they do not flatten out well interesting pictures are gained; one looks, for instance, into the concavity of the cup the sides of which have contracted toward the centre and become almost parallel, enclosing an hour-glass-shaped orifice, the base of which is so thin that it seems absent. In the well-made specimen the cells lie perfectly flat and are quite round when alone, polygonal when in contact. Sooner or later near the edge of the specimen crenated cells are seen; that is, they become spherical and covered with small prickly points, giving the picture of a thorn-apple. These should never deceive, and yet they may in case a corpuscle presents but one of these projections, and that on its flat surface, in which case it is often mistaken for a small ring form of the malarial parasite. By *crenation*, however, is meant not alone this artefact of prickly formation, but a change in the contour of the corpuscle; that is, instead of a round disk with an even, circular margin (unless, of course, the corpuscles are crowded one against the other), the margin is uneven and shrunken, as is seen for instance in quartan and æstivo-autumnal malaria.

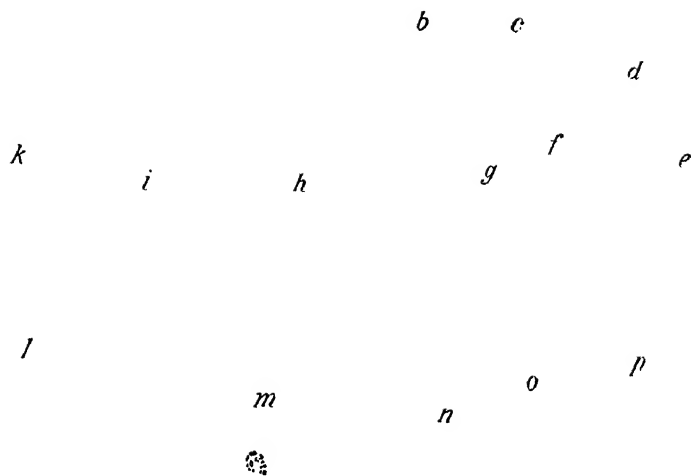
The presence of *poikilocytes* should at once attract attention. By these are meant corpuscles which are abnormal in the shape of the cell as a whole (not the fine irregularities of crenation). Poikilocytes may be due (1) to technique. If the cover-glass be pressed upon, a certain number of the corpuscles will fragment into small spherical masses and small elongated rods which resemble bacilli. The ease with which this fragmentation occurs depends to a great extent on the condition of the corpuscles. If, because of disease, they are "weak," a slight injury which would not affect a normal corpuscle will cause them to break up (Stengel). In case the cover-glass has been moved after the cells have spread, they will suffer considerable distortion. (2) A specimen of fresh blood in a very warm, moist chamber, and especially if at 50° to 54° C., will present the most remarkable picture. The corpuscles lose their shape and show definite contractile movements; some will elongate considerably and move around with a vermicular motion. We have known of a

whole hospital staff studying with astonishment the gyrations of these overheated red blood-cells, sure that some new parasites had been discovered. More commonly the corpuscles under these conditions are seen to bud, and these buds to become detached and swim in the serum as microcytes. Sooner or later in such a specimen nearly all of the poikilocytes will fragment. (3) Age. When the specimen is made the corpuscles may be considered living cells, but in three or four hours death processes are visible; these appear much earlier in the blood of a diseased case. They are studied best in well-sealed specimens on a warm stage, and resemble those of the overheated specimen, except are less in degree. (4) But the poikilocytes which interest us most are cells which are misshapen when the specimen is first made, and which probably were so while in the circulation. A very few may be found in fresh normal blood. They occur in any severe anæmia, but especially in primary pernicious anæmia of even mild grade. Of the many forms, two were once supposed to be characteristic of this disease, those resembling a battledore, and the elongated or sausage forms. Poikilocytes certainly seem to have amoeboid motion, at any rate, they are masses of contractile protoplasm. This is particularly seen in the small microcytes which can change their shape considerably and rapidly. (Plate I, 23-28; Fig. 93, k.)

The elasticity of the cells certainly varies. The large pale cells in anæmia look flabby; poikilocytes may be cells which were round in the circulation but with diminished elasticity, hence when the smear is made lose their shape; in lead poisoning it is said to be increased.

In the budding cells so often seen the projections may have the color of the normal cells or be paler or darker. They are attached by a longer or shorter pedicle, and often break free. Such buds which break loose and which have no hæmoglobin are supposed by many to be illustrations of the formation of some blood-platelets.

The size of the red blood-cells should be noted. Normally in the adult the average diameter is 7.5 microns. The student should carefully observe the variations in size, and try to determine which small cells are preformed and which the result of his technique. The size of the cells, so uniform in the adult (and yet a few dwarfs will be found), varies much in the normal infant blood. In certain diseases also the variation is considerable; in some, as in chlorosis, there is a quite uniform diminution; in other diseases, as pernicious anæmia, the majority of the cells will be larger than normal; in secondary anæmias the size varies, large, small, and normal sized cells being present, true also of pernicious anæmia, but to a less degree; in tertian malaria it is often the size of the cell which helps us to find the parasite, a large, swollen, pale cell attracting attention to the enclosed tertian form, while a small shrunken cell may point to a



*H Becker*

FIG. 93.—Fresh blood. *a*, cells with Maragliano's endoglobular degenerations; *b*, cell containing a navicular body, from a case of measles; *c*, the bacillus-like degeneration; *d*, a Maragliano degeneration in process of extrusion; *e*, a form of "hæmoglobin degeneration" giving a dark area; *f*, like *a*; *g*, like *e*; *h*, a degeneration like *e* but almost free from cell; *i*, a pseudo "segmenting parasite"; *k*, an "amœboid" microcyte; *l*, æstivo-autumnal hyaline malarial parasites; *m*, a full-grown æstivo-autumnal parasite, and, *n*, a segmenter, both found in the peripheral blood; *o*, same as *l*; *p*, macrophage from a case of pernicious malaria filled with malaria parasites.  $\times 900$ .



quartan or æstivo-autumnal, although in the case of the quartan the pigment and the protoplasm itself will probably first attract attention. The average size is said to be increased in jaundice, cholera, lead poisoning, and leukæmia; also in congenital heart disease and in cretinism.

COLOR.—The color of the corpuscles, normally of a greenish-yellow, may vary either in depth or in shade. Variations in depth of the color depend on the amount of hæmoglobin, and the trained eye will even suspect the color index from the examination of the fresh blood. Often by focussing the biconcavity is seen in normal corpuscles, but is not very evident, and in many cells cannot even be imagined. In case of a "light-weight" corpuscle, however, it is much more prominent; the corpuscles often appear to have no centre, and even a narrow ring may be all that is seen, the so-called "pessary forms." In other conditions the corpuscles seem to lack all biconcavity, and some may appear even biconvex, especially true of some microcytes.

The change in color tone is of particular interest. This seems to be due to some chemical change of the hæmoglobin. The corpuscles containing the quartan and especially the æstivo-autumnal parasites are beautiful illustrations of this, some cells which contain even a young ring-form appearing much darker than the other corpuscles and of a greenish "brassy" tone. A similar although less marked change in color is seen in microcytes and in cells fragmented by mechanical injury. The color of cells and fragments of cells seen in macrophages and phagocytes is so markedly changed, often so very green, that it is hard to believe it to be due to hæmoglobin.

In some diseases there is a quite uniform change in color. In chlorosis, for instance, nearly all of the cells are paler than normal; in pernicious anæmia a large number will seem darker than normal. In other conditions there is more variation in the shade of the corpuscle, as in secondary anæmia and in malaria. It is our custom each year to distribute to the class fresh specimens from several cases of chlorosis, primary anæmia, and secondary anæmia, and ask the students to decide from the appearance of the corpuscles from which condition the blood was obtained. They are required to make a fresh specimen of their own blood for comparison. Soon the students are able to express a pretty definite opinion concerning the nature of the case.

NUCLEATED REDS are sometimes very easy, but often very hard, to find in the fresh specimen, the nucleus being indistinct. To any one who has studied amphibian blood this is not at all surprising, since in that blood in which every corpuscle is nucleated one is often surprised at the small number of nuclei he really sees. An occasional normoblast is seen in normal blood, but it is a pure anomaly.

The DEGENERATIONS of the red blood-cells are very important (Fig. 93). These are necrobiotic changes, which appear sooner or later according to the intravascular condition of the blood, or the treatment it receives when or after the specimen is made. The "total" degenerations have already been described. More important than these are those partial degenerations which go under a variety of names, as vacuolization, pseudo-vacuolization, pseudo-nucleation, *état cribriform*, globular decolorization, and more commonly Maragliano's endoglobular degeneration, or, in short, "Maraglianos." These Maragliano endoglobular degenerations are seen in normal blood usually from thirty to seventy minutes after the specimen is made. Usually they are found in the centre, but may be near the periphery of the cell. A cell may contain one or several. At this point the corpuscle appears thinner and a vacuole-like area appears which seems free from hæmoglobin (and the surrounding plasma stained). This area is usually round, although it may be elliptical, and increases toward the periphery until a mere rim of hæmoglobin-containing protoplasm may be left. Although they resemble vacuoles, they are probably areas of coagulative necrosis. These degenerated masses certainly change their shape and their position within the cell; they are extruded sometimes from the cell or remain when this goes to pieces. Whether their changes in shape are due to contractions of this degenerated protoplasm or to those of the surrounding normal protoplasm is a hard question, but the rapid motions that they make may lead the beginner to suspect that they are malarial parasites. It is not true amœboid motion, since it is not through their change of shape that they change their position. The rapidity with which these appear in the specimen, other things being equal, depends on the intravascular condition of the corpuscles. Maragliano and Castellino have used the time of the appearance of these degenerated areas as a basis to group diseases and stages of disease, giving to it a certain prognostic value. They may have laid too great stress on these degenerations, but it is certain that in almost any severe disease, and especially in the primary anæmias, they appear much earlier than in normal blood. The best description of these degenerations is that given by Maragliano and Castellino.<sup>1</sup> These vacuole-like areas may be mistaken for nuclei and for malarial parasites. The latter mistake, one may be sure from specimens sent to the clinical laboratories, has been responsible for several "unusual cases" of malaria which have been reported, in which it was claimed only hyalines were found. Although only the trained eye will recognize these, it may be said, in the first place, that their size differs from that of the parasite, beginning smaller and soon becoming larger; that they grow more numerous the longer one searches for them, so that the

<sup>1</sup> *Zeitschr. f. klin. Med.*, 1892, vol. xxi.

student is often surprised that he should have overlooked so many "parasites" at first, since he finds them so easily later; they occur, as a rule, in the centre of the cell, although this is not at all constant; they are round or oval in shape, and, what is most important, they look more like vacuoles with very sharp edges, although not much more so than does the ring form of the hyalines; on changing the focus up or down this vacuole-like area enlarges or diminishes in size, while the parasite becomes more and less distinct; in general they are much easier to see than is the parasite; their movements may be similar to those of an amœboid organism, and their periphery may show the same wavy motion, but true amœboid motion is not present; they change position and they change shape, but they do not accomplish the former by means of the latter. Beautiful "segmenters" may be seen (see Fig. 93, *i*). In Fig. 93 the attempt was made to show these differences (contrast *a*, *f*, and *o* with *l*). In fixed specimens they show a granular structure and will take a basic stain, but have no red chromatin mass.

Maragliano considers that many so-called nucleated reds are really nothing but these degenerated cells; but the differences in size, the changes in appearance of these on changing the focus, and the distinct chromatin net-work of the nucleus should not allow this error.

Various other areas deserve particular notice; for instance the oval or ring forms, which are of the shape of a hyaline malaria ring with a circular refractive centre; these sometimes have a definite crescent shape, and are famous since once described as the parasite of measles, and more recently as that of spotted fever (see Anderson's figures). What these areas are is not clear; they change shape in a peculiar way; they do not increase in number or grow larger on standing; they occur in the greatest variety of conditions, but especially in measles (see Fig. 93, *b*).

In other cells are rod-like areas resembling bacilli, *c*. These "bacilli" may keep up a constant vibratory motion, moving practically through the whole substance of the cell, and hence the mistake sometimes made of suggesting cultures to isolate this organism is not to be wondered at. We remember one physician who was confident that these were typhoid bacilli within the cells. Others speak of them as "splits" in the cell.

Another common degeneration presents the appearance of a small cell on top of a larger and paler one, since the latter presents a dark circular area, but focussing shows them in the same plane (see Fig. 93, *e*, *h*, *g*). As a rule, this is an illustration of Ehrlich's hæmoglobinæmic degeneration, areas of condensed protoplasm, hæmoglobin separated from stroma; although superimposed cells do occur. Another example of this degeneration is in æstivo-autumnal malaria; cells are

seen in which the hæmoglobin seems to be gathered in a mass around the parasite (see Plate V, o). This is also seen in nucleated red cells which have the appearance of a microblast lying on a macrocyte. It is often best seen in stained specimens. This degeneration may explain some of the "acidophilic granules" of the red cells which have been described. This degeneration is best seen in cases of pernicious anæmia.

The granules studied by Vaughan<sup>2</sup> are to be observed in the fresh blood. The skin of the finger is well cleaned with alcohol and ether, and on it is placed a drop of Unna's polychrome methylene blue. The skin is pricked through this drop, hence the blood comes in contact first with the stain. A drop of the blood thus mixed with stain is transferred to a slide and covered at once with a cover-glass. In a few minutes a few cells are seen to contain granules staining violet. These granules are coarse or fine, sometimes in a line across the cell, sometimes connected by a filament. Their occurrence is remarkably constant; in normal adult blood they are present in 0.5 to 1.8 per cent. of the red cells; and in almost exactly this percentage of cells in a great variety of diseases with little influence on the blood; in the blood of new-born, in 1 to 7 per cent. of the cells; in that of a foetus, two and a quarter inches long, in 24 per cent.; in anæmias they are increased, in the primary pernicious occurring in even 18.8 per cent.; in general their number is parallel to that of the nucleated red cells. Vaughan gives good reasons for thinking that these are remains of the nucleus; they are not artefacts, and occur especially in normal-looking cells, in the position of the nucleus, and in conditions in which nucleated reds occur. He suggests them as a more delicate sign of anæmia than nucleated reds.

Morris\* has called attention to granules which are almost certainly nuclear fragments, and which are found in the blood of the human embryo, in the anæmia of infancy, and in those conditions of the adult in which other and clear evidences of blood degeneration are present, as pernicious anæmia, secondary anæmias, chronic myeloid leukæmia, etc. These granules, which are nearly always round and single, are sharply circumscribed, and eccentrically placed, and they have the same staining reaction as the nucleus.

**Leucocytes.**—The presence of a leucocytosis and its character will often be suggested by the fresh examination. The most of the surface of the specimen should be examined before forming a definite opinion of their number, since the distribution of these cells varies somewhat. Especially is this true of the so-called "stroked" specimens made by drawing the drop along the slide by another slide, a spreader, or a piece of paper.

<sup>2</sup> Jour. of Med. Research, 1903.

\* Arch. of Int. Med., March, 1909.



The leucocytes will be found as colorless, nucleated, amœboid or immobile cells, which do not float in the current.

**Lymphocytes.**—These are in size equal to, larger, or smaller than a red blood-cell; the nucleus is relatively large, round as a rule although sometimes deeply notched, and central in position; the protoplasm is scanty, sometimes hardly seen, in other cases presenting a ragged edge around the nucleus, and may appear somewhat granular; on the cold glass these cells of normal blood are not seen to move. Normally they make up from 22 to 25 per cent. of the leucocytes. On a warm stage those in lymphatic leukæmia especially are said to be amœboid.

**LARGE MONONUCLEARS.**—These when typical are two or three times the size of a red blood-cell. The nucleus is often round, but more often oval in shape and eccentric in position. The protoplasm is very abundant and is clear. Although these cells appear non-amœboid, yet it is interesting that in malaria they are the dominant phagocytes. They are about 1 per cent. of the total number.

The “**TRANSITIONAL CELLS**” of Ehrlich seem to be old forms of the large mononuclears. They are the largest of all cells. The nucleus is pale and often deeply notched, giving it the so-called “saddle-bag” or the wallet-shape. The protoplasm is abundant. In some cells may be seen a few granules in the proximity of the nucleus. These constitute from 1 to 3 per cent. of the leucocytes.

**POLYMORPHONUCLEAR CELLS.**—*The finely granular cells of Max Schultze* constitute from 70 to 72 per cent. of the total number. They are from 10 to 15 microns in diameter, the size depending chiefly upon the extent to which the spherical cell is flattened out against the glass; the protoplasm is clear and filled with fine granules of a dust-like character; the nucleus has the shape either of a bent rod, a skein of fibres, or, as a rule, there seem to be several masses of chromatin matter, hence the old name polynuclear cells. These when they leave the blood-vessels are the ordinary pus-cell and are the greatest phagocytes of the body.

The *coarsely granular cells of Max Schultze* (eosinophiles) are usually a trifle smaller than the preceding. The nucleus is usually more regular, but this feature is not constant; the protoplasm is filled with coarse, blackish, very refractive granules of quite uniform size and shape, being round or slightly oval and about 1 micron in diameter. These are the most amœboid cells of the blood, and make up from 2 to 4 per cent. of the leucocyte count.

The *Mastzellen* in the fresh specimen resemble the finely granular cells. They cannot with any certainty be recognized, and yet will often be suspected. The granules are more irregular in size, some quite as large as of the coarsely granular cells, and do not fill the pro-

toplasm quite so completely; the nucleus is often trilobed. These cells are present in the normal blood to the extent of from 0.5 to 1 per cent. of the total number.

In various blood specimens the size of the leucocytes will seem to vary considerably. This depends upon the thinness of the smear, and hence the extent to which the leucocytes have flattened out. In the thick parts of the smear they will appear small, since spherical; in the thinner parts large, since flat.

PIGMENTED LEUCOCYTES containing blood pigment are best studied in the fresh or air-dried specimens. This pigment may be melanin, blackish or brownish granules in which no iron can be demonstrated, formed within the malarial parasite, and when set free picked up by the leucocytes. These are very important in the diagnosis of malaria. Similar granules are seen in the leucocytes in cases of melanosarcoma and then indicate a generalization of the tumor.

Hæmosiderin pigment occurs rarely in the leucocytes in cases with rapid blood destruction as ochre granules. The iron may be demonstrated by treating the smear first with 2 per cent. potassium ferrocyanide, then with 0.5 per cent. hydrochloric acid. The specimen is mounted in glycerin. The granules become blue in color.

**Müller's Blood-Dust.**—Blutstäubchen, or Hæmokonien granules. Müller called attention a short time ago to the presence in the normal blood of very fine granules which danced actively between the corpuscles. Finding a large number of them in a case of Addison's disease he supposed they bore some relation to that malady, but later decided that they were present in all bloods, although in very varying amount. They are described as small round colorless granules, which vary considerably in size, some one micron in diameter, but for the most part very fine and dust-like. The larger ones resemble micrococci. They are best seen by gas-light. Their nature Müller could not determine, but since they did not give the osmic acid test he said they were not fat, although they resembled it, and as they were not cleared by acetic acid he decided they were not of albuminous nature.

They were further studied by Stokes and Wegefarth,<sup>3</sup> who decided that they were the extruded granules of leucocytes. The reason for this opinion is that they resemble these granules in size, in man being both coarse and fine. Good additional evidence is furnished by comparative anatomy, especially the horse and rabbit, which animals have peculiar granulations in the leucocytes and similar blood-dust granules; they can be seen to escape from the leucocytes if certain reagents are added to the blood; and, lastly, the larger ones take an eosin stain. In the stained specimen the free granules are easily seen. Their relation to immunity, which point particularly interested these writers,

<sup>3</sup> Johns Hopkins Hosp. Bull., December, 1897.

does not concern us, but from the study of fresh and dried blood the origin they suggest seems very probable for a certain number at least of these granules. It is probably these which have been described as spores of certain parasites in the blood.

The fat of the blood is evident in the fresh specimens as exceedingly fine dust-like granules which would escape observation if they were not carefully looked for. These granules form a perfect cloud in the plasma in cases of lipæmia.

The **platelets** are seen either singly or in large masses, or as masses of granules in the periphery of which are vacuole-like areas containing a watery fluid, the so-called "granular masses of Max Schultze." To one point we would call especial attention. In the fresh blood specimen all platelets will stick to the glass or to the corpuscles, and any floating fragment of protoplasm is certainly not a platelet, however much it may resemble it. (See page 569).

The fresh specimens are the best in which to study the large **macrophages**, enclosing malarial parasites, red cells, and cells containing parasites in malaria, and very many red cells in typhoid fever. These cells are very poorly preserved in stained specimens. (See Fig. 93, *p.*)

In pregnancy **placental cells** (syncytium) "are commonly found" (Veit) in the mother's blood, perhaps being swept off in the blood-current.

The **fibrin net-work** should be looked for. The fibrin strands are often seen radiating from

small masses of platelets. The amount is very large in certain diseases, as in pneumonia, acute articular rheumatism, *et al.*

**Counting the Red Corpuscles.**—The instrument to be recommended is the **Thoma-Zeiss** (see Fig. 94), which we have found uni-

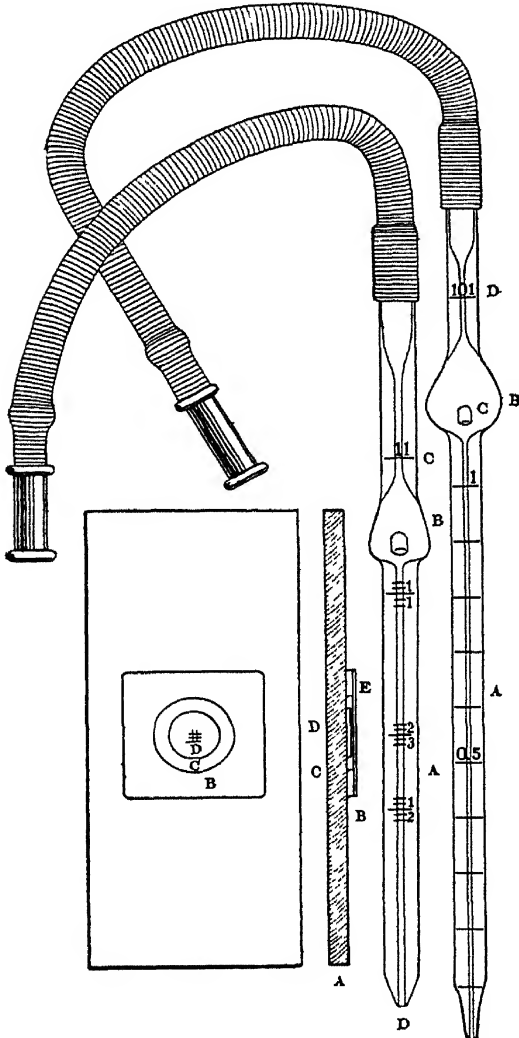


FIG. 94.—Blood-counter (Thoma-Zeiss). To the right is the ordinary form of pipette for red cells; the other is a leucocyte pipette with improved markings and point, D. The ruled counting chamber is shown on edge and face view. A, the slide; B, the ring; D, the ruled table and C, the "ditch;" E, the cover-glass.

formly good. In this clinic we have in constant use fifty blood-counters, and can say that the Zeiss goods never disappoint us. We have tried slightly cheaper makes, but have always been obliged to discard them. For blood-counting the best instrument is none too good.

The blood-counter consists of a PIPETTE for mixing the blood to a certain dilution, a counting chamber by means of which a layer of known depth and area is obtained, and a special cover-glass to serve as the upper boundary of this layer. The pipette is a graduated capillary tube (Fig. 94, *A*) opening into a dilatation, *B*, at the opposite pole of which empties a second shorter glass tube, *D*, to which is attached a rubber tube with a mouth-piece. This pipette is so graduated that the capacity of the reservoir to a line on the shorter tube, marked 101, is exactly one hundred times the capacity of the capillary tube from its point to a line marked 1. As a rule, the unit on the long tube is divided into ten parts, an unnecessary provision. Much to be preferred are those pipettes which have on either side of the 0.5 and the 1 marks, two smaller marks, each indicating  $1/100$  the length of the tube (see page 521). The end of the long tube should be obtuse, as *D*, of leucocyte pipette, since in the quick movements made it is easy to break this point. In the dilatation is a small glass ball, *C*, which aids much in mixing the blood with the diluting fluid. The pipette is to be cleaned by washing out first with water, then with alcohol, and then with ether. Air is then sucked through, not blown, until the bulb is visibly clean and on rolling the tube the glass ball rolls freely within it.

Boggs \* has improved the pipette by inserting in the rubber tube a Wright's "throttled capillary." This consists of a capillary tube which has been heated in a very small flame and then quickly drawn out into a fine thread. The calibre of this tip must be so fine that when gentle suction is made the air comes through very slowly. The outer, protecting tube is from 5 to 7 mm. in diameter and drawn out to an hour-glass shape. The large part of the capillary is marked with a file, so that it may be conveniently broken off after it has been cemented in the holder. The cementing is easily done by molding a little sealing-wax near the throttled end, passing the larger, free end of the capillary first through the holder, and, after warming gently at the constricted part, drawing the waxed end down into the narrow waist of the tube. The wax softens and fills the neck of the hour-glass tube, and on cooling leaves the capillary firmly cemented in place. Each end should be about 5 mm. shorter than the container. It is

\* Jour. of A. M. A., January 5, 1907, vol. xlviii, p. 47.

easy to break off this larger end of the capillary at the point marked, by using a fine forceps passed into the larger tube.

With a little patience any one can make the apparatus. A few trials may be necessary in order to determine the fineness of the "throttle." This should be so arranged that with gentle suction the filling of the pipette is slow. The device should be connected with the pipette with the fine point toward the mouth-end.

In the subsequent washing of the pipette with water, alcohol, and ether it is advisable to remove the controlling device, as a drop of fluid drawn into the "throttled" end will seal it and render it useless.

This controller makes it easy to draw the column of blood steadily and slowly to the point desired, and it also keeps the blood from falling from the pipette when the tip is transferred to the bottle of diluting fluid.

The worker will save much time by paying careful attention to his pipette. It should always be thoroughly clear before any attempt is made to use it. The capillary tube should contain no trace of clotted blood; the glass ball should roll freely in the bulb; the rubber tube should be very flexible and not cracked near the mouth-piece, and it should contain no saliva.

The DILUTING FLUIDS used are several in number. The one commonly used is Toisson's, the composition of which is

Water (distilled), 160 cc.;  
Glycerin (neutral), 30 cc.;  
Sodium sulphate, 8 gms.;  
Sodium chloride, 1 gm.;  
Methyl violet, 0.025 gm., or just enough to give the desired tint

Hayem's fluid is preferred by some:

Distilled water, 200 cc.;  
Sodium chloride, 1 gm.;  
Sodium sulphate, 5 gms.;  
Mercuric chloride, 0.5 gm.

Sodium chloride can be used in rather strong solution (3 per cent.); but it is probable that the physiological 0.6 per cent. solution will lacerate a certain number of corpuscles.

These fluids must always be fresh and recently filtered, since yeasts certainly do grow in those not containing an antiseptic salt, and these yeasts repeatedly lead to error.

The COUNTING CHAMBER consists of a heavy glass slide, *A*, on which is cemented a thick glass ring, *B*, the surface of which is beautifully polished. This ring surrounds a circular table of glass, *D*, the height of which is just 0.1 mm. less than that of the surrounding ring, and upon this is the ruled area. Between this glass table and the inner

edge of the ring is a small ditch, *C*, to catch the drop which may run off from the table and prevent its running up between the ring and the cover-glass on the other side of the ditch. On the central glass table are ruled twenty-one parallel lines, 0.05 mm. apart. Crossing these at right angles is an exactly similar set of lines. The result of their intersection is a 1 mm. square, divided into 400 equal small squares (see Fig. 95). Through each fifth row of squares is ruled an extra line. This extra line is not a boundary, but merely aids the observer to keep his position in the ruled area. Indicated, not bounded, by these extra lines, the square millimetre is divided into sixteen units of twenty-five small squares each.

In choosing a blood-counter the lines should be carefully studied, since certain makers have put on the market very imperfectly ruled slides. They should first be examined dry, to make sure that the lines are complete, and then covered with a drop of water that their sharpness may be determined; for we have seen lines which appear very distinct on a dry slide practically disappear when a drop of water covered them, since the distinctness of the line when covered with water depends not on its depth and width, but on the sharpness of the edges. If this little point is borne in mind, there will be much less dissatisfaction with blood-counters.

Before use this counting cell should be well washed with water and carefully wiped, care being taken that no lint be left on the surface of the glass ring. Alcohol and ether should never be used, since the centre glass table is cemented to the slide and is easily loosened by these reagents.

The COVER-GLASS is a heavy one with planed surface, made particularly for this use. Ordinary cover-glasses can never be used, for they are of uneven surface; they are also cut from a sheet of glass often not well flattened, and hence will not lie parallel to the surface of the glass table; and lastly, they are so thin that the capillarity of the drop will bend them down slightly.

We have not yet had opportunity to use the Bürker chamber, but from the description given of it one would infer that it was an improvement on those in common use. Sahli describes this chamber on page 859 of the fifth edition (1909) of his *Lehrbuch der Klinischen Untersuchungs-methoden*.

DILUTING THE BLOOD.—After the ear, for example, has been pricked and the blood flows freely, a large drop is allowed to collect, which it should do rapidly, and is drawn into the pipette to the mark 0.5 or 1 according to the nature of the blood. For normal blood it should be drawn only to the point 0.5, in anæmic persons to the point 1. Before drawing in the blood the instrument should be tested to make sure that there is no obstruction in the tube. The blood is rapidly drawn exactly to the line desired. This will require considerable experience. If drawn too far the column may be shaken down

somewhat by tapping against a towel or rubbing it against the end of the finger, but unless there is very little correction to be made the instrument would better be cleaned up again and the whole work started anew. For this reason we prefer those pipettes with the extra marks indicating  $1/100$  the length of the tube, since if the column does not reach exactly the mark desired, it can be drawn to one of the other marks and then the necessary correction made. For instance, if drawn two marks beyond the 1 the worker should proceed and then diminish his final result by 2 per cent. Considerable error arises if the length of the blood column is not just right. With the long form of pipette which we use the error of 1 mm. means an error

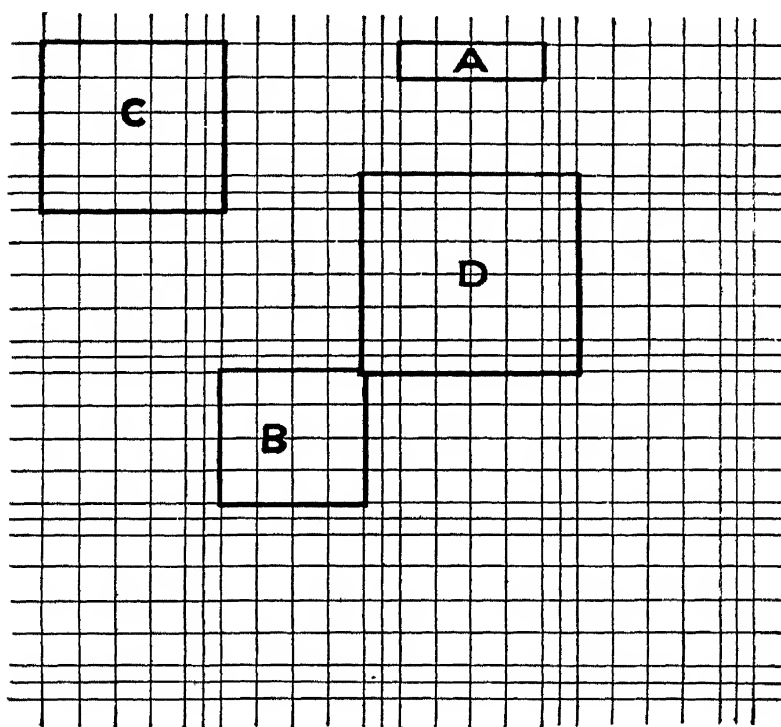


FIG. 95 — The one square millimetre ruled area, much magnified, showing the units in common use.

in our result of 2.2 per cent., and in the more common short model of pipette, one of 2.6 per cent. The mistake of 1 mm. should not be made, but it is very easy to make one of 0.5 mm., and this error of 1.3 per cent. is too great to disregard. (For these figures it is supposed that a red blood-count with the blood drawn supposedly to the 0.5 mark is being made.) After the column is at the right height the tip of the pipette is then cleaned, either on the finger or by wiping it on a towel, and the pipette plunged into a bottle of the diluting fluid. The diluting fluid is now drawn into the pipette, the tube being held vertically and rotated between the finger and thumb while the fluid enters. By this rotation the diluting fluid mixes at once with the

blood as it enters, and hence a layer of pure blood does not rise on the surface of the fluid and pass into the small tube undiluted; also in this way can best be avoided the bubble of air which often clings to the inside of the bulb. The fluid is aspirated until the mixture reaches the mark 101. It is not so necessary to accurately reach this mark, since a difference of 1 mm. in the case of the instrument now before us would mean a negligible error of only 0.03 per cent. The pipette is now withdrawn from the diluting fluid, the thumb placed over its point, and then the upper end closed by the first finger. The rubber tube may be removed or not as the worker desires. The pipette is then vigorously shaken for at least one minute. It is to be shaken in all axes except perhaps directly in the long axis of the tube, which would allow a small number of corpuscles

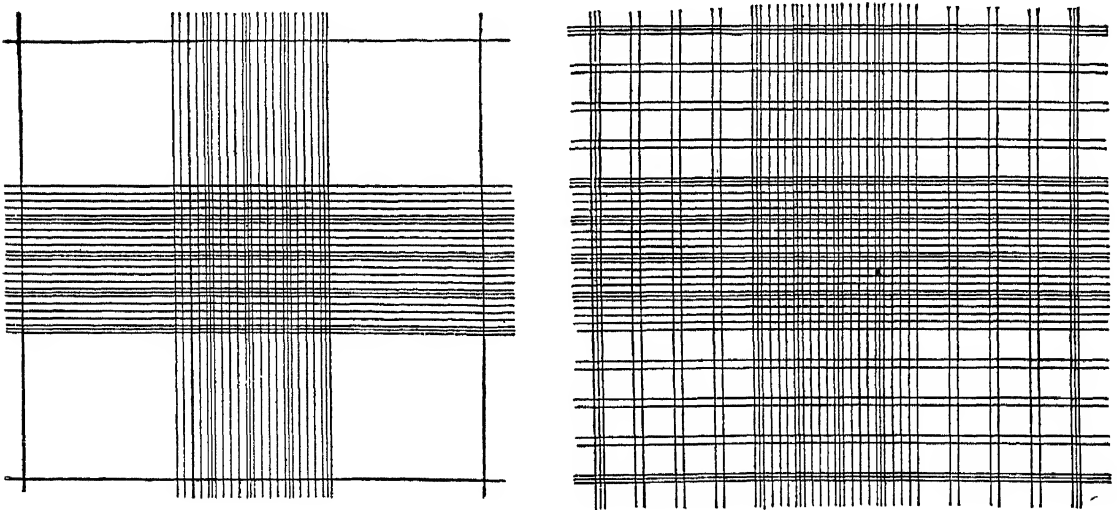


FIG. 96.—A, Zappert ruling; B, Turk's ruling.

to be shaken into the column of fluid in the capillary tube, which, of course, contains no blood. Then at once two or three drops are blown out in order to remove that column of fluid which has not entered into the mixture. It is well now to let the pipette rest for about ten minutes in order that the leucocytes may become stained by the methyl violet of the Toisson's fluid. When, however, the pipette is picked up again, the shaking should be repeated as vigorously as before. If the blood is not to be counted at once, the pipette may be sealed by stretching a rubber band over its ends. At the end of several days it may be shaken again after the fluid in the long capillary tube has been first blown out.

**Filling the Cell.**—After shaking again and blowing out two or three drops, a small drop, the size of which can be learned only by practice, is blown out upon the ruled table and then covered at once



by the cover-glass. The drop should not be so large as to run down into the ditch at any point, and should be large enough to almost cover the glass table. A large drop which runs into the ditch or too small a drop which merely covers the ruled surface will in either case introduce a certain error. The cover-glass should be put in position at once. The best way to do this is, we think, to grasp it by two diagonal corners, to place a third corner against the slide with the edge of the glass ring as a fulcrum, and to hold it in that position by a finger of the other hand. It is thus held up away from the drop by the edge of the glass ring as a fulcrum. By now raising the finger the cover is rotated onto the drop rapidly, and also in such a way that no air-bubble is left, which so commonly occurs if the cover be merely dropped on.

The next step is to determine whether between the cover-glass and the glass ring is any dust or dirt, which, of course, increases the thickness of the layer of diluted blood. This is done by holding the slide almost on a level with the face and toward a window, and in such a position that the light is totally reflected from the surface of the cover-glass. If the cover and slide are in good apposition, a beautiful band of colors should cover the surface of the glass ring, due to the phenomenon of interference of light so beautifully seen in superimposed layers of thin glass. Should these colors not be there the cover-glass may be touched by some instrument, but not by the point of a pencil which leaves a small amount of carbon on the glass. This may bring out the color bands, and if they remain the specimen is satisfactory. If, however, around the point of pressure appear the concentric Newton's rings, and these disappear when the pressure is removed, this slide should be cleaned up and another trial made. Certain workers seem to think that it makes but little difference if this phenomenon of light interference is not present. We consider it a very important point in the technique. When not present it usually requires considerable pressure to bring out five concentric rings, and we know that at the fifth ring the space between the cover-glass and the table is 1.4 microns, which is the thickness of one corpuscle. Over the rest of the surface, therefore, this distance must be considerable. Another method of getting the necessary contact is to allow a drop of the fluid to run under the edge of the cover, and then squeeze with the fingers the cover hard down on the slide (note the Hayem method). Dr. Cabot says that some put four small drops of the fluid, one for each corner of the cover, on the slide before the cover is put on.

This phenomenon of light interference is to many a great bugbear. It should not be so. We find it is very easy or very difficult to obtain. In the case of a well-made counting chamber it is so easy that one

attempt usually is sufficient, and from the clean appearance of the slide before the cover is put on one can predict whether or not the bands will appear. We have bought, on the other hand, counting chambers with which the bands could almost never be obtained. In handling the glass slide it should be kept as horizontal as possible, since a slight tilting may allow the cover-glass to slide off. If the cover be sealed by the fluid this will not happen. The counting chamber should now be allowed to rest for from three to five minutes that the corpuscles may settle down onto the surface of the glass, and hence render much easier the counting than if considerable focussing is necessary in order to bring them all into sight.

It will be seen that at certain points of the technique the movements must be very rapid, and it is no exaggeration to say that greater mistakes are sometimes made by too careful than by too quick work; since in trying to avoid slight errors greater ones are committed. The points of greatest importance are, the rapid filling of the pipette, and the rapid dilution with the fluid; if considerable time be spent doing this, on the slide later will be found groups of corpuscles not broken up by the shaking; the shaking must be thorough, and the drop of blood blown onto the counting chamber must be blown out at once after the shaking, and from the fluid in the interior of the bulb, not that from the capillary tube; lastly, the least possible time should elapse in putting the drop on the ruled table and covering it with the cover-glass. It is interesting to note that with the two instruments used by the French—the Hayem and the Malassez—are introduced two errors which we constantly try to avoid with the Thoma-Zeiss. Hayem recommends that an extra drop of the blood be allowed to run between the cover-glass and the table in order to seal the specimen against evaporation. Should a drop of the fluid in the case of the Thoma-Zeiss run from the ditch up between the glass ring and the slide, we would insist that the slide be cleaned up and the work done over again, yet others do not agree to this point, and think that thus is the light phenomenon more easily obtained. In the Malassez instrument is a mechanical contrivance for holding the cover-glass at a distance of 0.1 or 0.2 mm. as may be desired.

The student should learn that it takes less time to clean up his counting chamber or his pipette and begin anew, than to count a lot of extra fields with the hope of counteracting some error which he is conscious of having made. If the worker has two slides the two can be used very conveniently, the one settling while the other is being counted.

The next point is of great importance. Before counting a single cell the whole of the surface covered by the blood, even to the edge of the table far away from the ruled area, should be carefully examined

with the low power, to make sure that the distribution of cells is even. If this is not the case, no matter how even it may appear over the ruled area, the slide should be cleaned up and another preparation made.

**Counting the Cells.**—The power to be used in the case of the Leitz microscope is, for beginners, the 6 objective and the No. I ocular. The cover-glass is usually too thick to allow of the use of a No. III ocular. Later on the student may be able to use a 3 objective and a No. III ocular. A mechanical stage is often of use, and yet it is better to train the fingers to do that work.

The unit of the ruled surface (see Fig. 95) to be used is a matter of individual preference. Cabot recommends a unit of thirty-six small squares, *D*; that is, a unit the four sides of which are rows of squares through each of which passes one of the extra lines. Simon prefers a unit of the sixteen squares, *B*, through none of which the extra line passes. Sahli recommends a unit of four squares, *A*, four of which units make up the unit recommended by Simon. We prefer a unit of twenty-five squares, *C*, on two sides of which are rows with the extra line. The reason we prefer this is that it is to this unit that the slide is ruled, and the calculation of the corpuscles is easier than with any of the others, with the exception of the Sahli, and also that there is no danger, in case we count several units on one slide, of counting any corpuscles twice, as in the case of the unit recommended by Dr. Cabot. We count usually the four corner units, of twenty-five small squares each, of one slide, and then clean up and in a new drop count the same. In this way we have counted the cells over one-half of a square millimetre. The other workers recommend a much larger number than this and all state that it were better to count four hundred small squares. In counting, cells which touch the upper and the left-hand lines are included in the unit, while cells which touch in any way the right-hand or the lower boundary lines are to be disregarded, even though all but an edge lies inside or outside the square. Since counting is usually made downward and to the right, there is less danger of counting the same cell twice. If the cell is exactly in the corner it will be necessary to remember whether that particular one has been counted once or not. The beginner should pay no attention to leucocytes, counting them as if they were red blood-cells. The reason for this is that in normal blood in eight units the probability is that but two leucocytes will be seen, an error of but 0.08 per cent., which is, of course, negligible. It is easily seen that a very high leucocytosis will introduce enough of an error to make it pay to strain the eyes to tell which are leucocytes and which are not, for although the methyl violet will stain the leucocytes fairly well, it will also stain a deep violet a certain number of red blood-cells, and for

beginners at least it is difficult to tell in many cases the nature of the cell. Hence it is better, in leukæmia for instance, to count all leucocytes with the reds, then the leucocytes with acetic acid, and the difference will be the red cells.

If the diluting fluid used be fresh or recently filtered everything seen may be counted as a cell. If spores are present they will appear like small mononuclears, and ridiculous counts due to this fact are sometimes reported. Many of the corpuscles will appear distorted, and in some anæmias very small cells are easily overlooked. They should all, however, be counted, since if the technique is good and the fluids clean only blood-cells will be seen. The high color index of pernicious anæmia has perhaps with reason been attributed to the fact that microcytes are overlooked.

The students will thus count eight unit squares each of twenty-five small squares, and the sum of these cells will be the number over  $\frac{1}{2}$  sq. mm. This in normal blood will be about 1250 cells. This multiplied by 2 gives the number of cells in 1 sq. mm. of a layer of blood 0.1 mm. thick; this multiplied by 10 will give the number of cells in a cubic millimetre of the diluted blood, and this multiplied by 200 (providing the blood was drawn to the 0.5 point), the number of cells in a cubic millimetre of the undiluted blood, the desired figure. In case, however, that any other units are used it takes longer to calculate the count, except in the case of the Sahli unit, which is easy since his is  $\frac{1}{100}$  of the area of the square.

The difference between the extremes of these eight figures, each the number of cells in a unit of twenty-five small squares, we do not allow in the work of beginners to be over twenty-five cells. The reason for this is as follows: With good technique the distribution of the cells will be such that the extremes of these eight figures will easily fall within that limit. If they do not, it is easier to clean up and begin over, than by counting more units, trying to offset the error of poor distribution; if they do, then it is a waste of time to count any more units. Hence by good technique at the first considerable weary counting is avoided. If one's technique is so good that he can always conform to this rule, then later he can safely report a blood-count counting only four units or even only one. He may safely do this in his private practice. In the work of the clinic, however, we do not accept such counts, although we are confident that they would be more accurate than the counts of one who has not by actual experiment learned his error and by practice corrected it, or of one who thinks it possible by counting sixteen units to offset known errors in technique.

Our rule for training the third-year men is as follows: They are to use this method until they consider themselves fairly proficient. They then count the blood of one case, usually their own, daily at the same hour on each day until the difference between two successive days is not 200,000 cells and the difference between the highest and lowest of the eight units for each day is not over twenty-five cells (a good counter will often have a difference of only thirteen or fourteen cells). Two hundred thousand cells means that we permit a difference of 4 per cent. We choose this figure not because 4 per cent. represents the error in counting, but to make due allowance for daily variations which certainly occur, and because if the two counts vary by no more than this we are sure that the error due to counting alone is less than 2 per cent. Some students attain this quickly. We have known of students, however, who must repeat this for from twenty to thirty, even sixty, times before their work was satisfactory to themselves or to us. At the end of this time they are very certain to learn wherein lies the error in their technique. It is of interest that very often it is because they are too particular and take too much time in certain steps of their work. If the reader considers that it must be an awkward man who would take thirty days to attain this accuracy, we can only say that those alone who have tested their own accuracy know how inaccurate they can be, and that some of the least successful are the most surprised to find it out. Our students are seldom guilty of reporting "rises" or "falls" of 100,000 cells, nor do they ever report a count of 4,750,600. The student who is able to conform to this rule has confidence in his technique, a confidence which is usually earned by work. He has discovered his error if any has existed, and has learned to save himself considerable eye-strain, for we know in clinical microscopy of no task more wearisome than the counting of a large number of units. Blood-counting requires considerable practice. Even the good workers after a vacation of a few weeks find that it is necessary to make trials once or twice before they are ready again for accurate work.

After the count is finished the slide should be washed with water only and the pipette rinsed first with water, then washed clean with alcohol two or three times, and then with ether twice. Air is then sucked through by mouth until the glass ball rolls easily. A suction-pump will save a great deal of trouble. The student must be careful to blow no saliva into the tube. If he draws in the alcohol before the blood is entirely removed, a precipitate will form. This may sometimes be removed by nitric acid or by filling the pipette with pepsin-hydrochloric-acid mixture and leaving it in the thermostat overnight. In case a clot obstructs the bore of the pipette, it may be dislodged with a horse-hair; we do not allow a fine wire to be used, for this will easily crack off the end of the tube.

As to the error inherent in blood-counting, we can only say the best workers have not considered it possible to count with less error than 2 per cent., and some are satisfied with 3 per cent. An error of 3 per cent. would mean that two men counting with equal accuracy the same normal blood at the same time would get results which differ by about 150,000 cells. Good workers, however, will often come much closer than this, and we know of no better way to stimulate students to attain good technique than by insisting that a certain number of them, the more the better, shall each of them with a separate instru-

ment count independently the same blood. We have seen this result in considerable extra practice.

**Other Methods of Blood-Counting.**—The method of **Hayem** has been used considerably, particularly in France. Hayem's fluid (see page 461) is used as dilutant.

Two cmm. of blood are measured in a small capillary tube which much resembles that of a Gowers hæmoglobinometer, and blown into a small beaker, into which has already been measured by a larger pipette 500 cmm. of the Hayem fluid. It is impossible to wash out the large pipette, and since it is found that about 6 cmm. remain in it, the blood is considered diluted 1:248. The blood is well mixed by means of a small glass rod. The counting cell consists of a glass chamber similar in some ways to that of the Thoma-Zeiss, but without any ruled table, the rulings being projected by a ruled chamber which fits into the substage of the microscope. The layer of diluted blood counted is a cube, each side of which is 0.2 mm., hence a volume of 0.008 cmm. He recommends that now a drop of the diluted blood be allowed to flow under the cover-glass, in order to seal the specimen. The number of cells found in this area multiplied by 248, and this by 125, will thus give the number of cells in 1 cmm. of undiluted blood.

**The Malassez instrument** resembles the Thoma-Zeiss in many ways. A similar mélangeur is used, and a slide with a ruled table, but the cover-glass is held by a mechanism which can be so adjusted that the layer of blood is either 0.2 or 0.1 mm. thick.

**The Oliver Hæmocytometer.**—This very ingenious and simple instrument is based on the principle that if blood be diluted by a fluid which preserves the corpuscles, and in a test-tube rectangular on cross-section and composed of a longitudinally striated glass, if through a suspension of opaque particles in such a glass tube a candle flame be observed, each striation in the glass will act as a lens projecting an image of the candle flame to the back wall of the tube. But this image can be formed only when the suspension is of a sufficient dilution to admit the almost unobstructed passage of the light rays. The blood is measured in a small self-filling pipette, and is washed into the tube by means of Hayem's fluid from a medicine dropper the tip of which is covered by a small rubber tube. By shaking the test-tube covered by the thumb the suspension of corpuscles is made quite uniform, but the thumb should be slid off in such a way that the drop of blood clinging to the skin will be wiped off into the interior of the tube. The tube is then held between the thumb and the first finger in such a way that these form a frame, thus eliminating extraneous rays. The tube is held close to the eye, which looks through the long axis of its cross-section at a small candle ten feet distant in a dark room. The dilution and mixing are continued until at a certain point a bright horizontal line, which is a row of images of the candle flame, is seen across the test-tube. This line appears first at the edges of the tube. The proper dilution is obtained when these two lines from the sides just meet at the centre. The tube is graduated into one hundred divisions, the 100 point being that dilution found by experiment necessary to give the end reaction in a blood of 5,000,000 corpuscles per cubic millimetre. Hence each division corresponds to 50,000 cells. The end-reaction is so sharp that students trained to it insist they can detect a variation of 12,500 cells.

It should be distinctly remembered that Oliver invented this instrument as a more accurate means of counting the blood than are the Thoma-Zeiss and similar methods. He did not invent it as a short cut for approximate blood-counting. He distinctly stated that it was not to be used in diseases accompanied by a large variation in the size of the red blood-cells. We are sure from the quite extensive use that the instrument has had in this clinic that his claim in the case of normal blood is correct, and that slight physiological variations can

be detected, which would fall far within the limits of the accuracy of the Thoma-Zeiss instrument, and the instrument is to be heartily recommended for such work. We have found, however, that in the blood diseases, as he has warned, the error is so great that the instrument cannot be used. But the clinician cares nothing about physiological variations of normal blood, and finding that it is of no use in the blood diseases, and that in the primary anæmias there may be an error of over 2,000,000 cells per cubic millimetre, he discards it as useless. We emphasize this because its use has been recommended as an approximate and easy method of counting the blood in all blood diseases, and would refer the reader to a paper by Baumgarten,<sup>4</sup> who emphasized the error arising from abnormal sizes of the corpuscles, and from the precipitate in the plasma.

**The Hæmatocrit.**—This at first promised to save considerable time and eye-strain by giving a fairly accurate determination of the volume of the red blood-cells, that is, of the hæmoglobin-containing protoplasm. The instrument is a modified centrifuge capable of very high speed. Some forms use a diluted blood; others the undiluted. In the second case in each arm of the centrifuge (see Fig. 97) is a small glass tube of rather large bore calibrated with 100 divisions. One of these glass tubes is inserted in a rubber tube with a mouth-piece and the blood drawn in until the tube is even more than full. This requires a very large drop. The finger, covered with vaseline, is then placed over the free end and then the rubber tube removed. The glass tube is inserted in the centrifuge, in the other arm of which is the empty tube to balance the machine, and at once as high a speed as possible obtained and maintained until the column of centrifugalized corpuscles does not decrease. Each division of the tube corresponds to approximately 100,000 cells. Multiplying the number of divisions by this will give an approximate blood-count and a fairly accurate estimation of the volume of the red blood-cells. This may be accurate in the case of normal blood, but as in the different anæmias in which alone blood counts are of great importance the corpuscles vary considerably not only in size, but probably in elasticity as well, it is not at all certain that they will always pack down to the same degree. At any rate, the instrument has not been very popular for blood-counting. As is so often the case, the method was used first considerably, then almost abandoned, and now is again coming into favor. Aspelin<sup>5</sup> centrifugalizes a blood diluted with Müller's fluid in a special pipette. The blood need not be used at once, since this mixture will keep for some time. He reads the leucocytes at the same time.

Capps<sup>6</sup> thinks the volume index important, and the numerator of this is determined with this instrument (the count is the denominator). He certainly has published some interesting results. We use the hæmatocrit to detect the presence of lipæmia, cholæmia, or hæmoglobin-

<sup>4</sup> Johns Hopkins Hosp. Bull., July, 1902.

<sup>5</sup> Zeits. f. klin. Med., 1903, Bd. 49, S. 393.

<sup>6</sup> Jour. of Med. Research, 1903, vol. vi.



æmia. In the latter case, however, it is only safe when the plasma is free to say that hæmoglobinæmia is not present. Should the plasma be stained red one is not at all certain that this was so before the centrifugalization, for the mechanical injury to the cells may have set free a certain amount of hæmoglobin. The instrument requires very rapid work. It must be set up in close proximity to the bed. It makes a very loud and disagreeable noise, and hence is not a very satisfactory clinical instrument. To determine the volume of the red blood-cells the sedimentation by gravity in tubes to which a small amount of oxalic acid has been added to prevent coagulation is much preferred by some.

In the use of the instrument the springs holding the glass tubes in place should be occasionally tested, and the cups in which the tube rests should have at their base a piece of soft rubber; also the vaselined end should always be the distal. If these precautions be observed, the blood should remain in the tubes, but very often the considerable centrifugal force forces the whole column out of the tube.

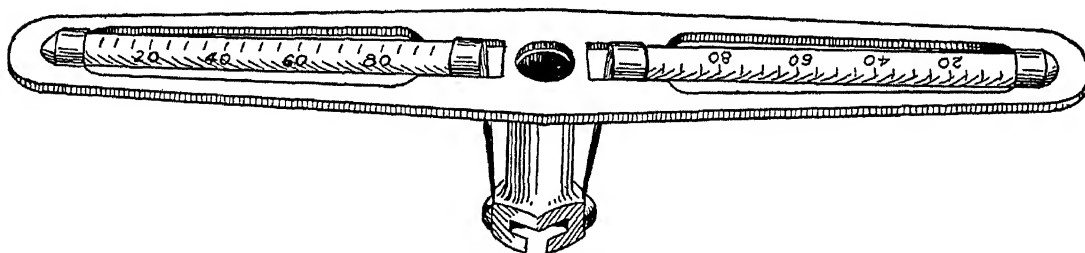


FIG. 97.—Arm of hæmatocrit.

A method which permits the specimens to be counted to be made permanent, that the enumeration may be made at leisure, is given by Strong and Seligmann.<sup>7</sup> The blood is diluted 1:100 for leucocyte counts, 1:20,000 for red counts, and then a quantity of known volume made into a dried and permanent mount. We judge it takes longer time than the ordinary method. Its results vary from 1 to 4 per cent. from those with the Zeiss.

**Leucocyte Counting.**—For a leucocyte count the same mixture in which the red cells were counted may be used, especially if Toisson's fluid was the diluent employed. In this case after counting the red blood-cells, the leucocytes over the whole square millimetre are counted. The trained eye easily picks them out, more from the difference in their refractivity than from any stain, since they are seen as bright cells when the focus is slightly raised. With the Thoma ruled slide the leucocytes in the whole millimetre field of eight separate drops should be counted. This requires about all the time at the disposal of the worker, and the number of cells counted is much too small, yet a fairly approximate result is obtained. It is much better

<sup>7</sup> Brit. Med. Jour., July 11, 1903.



to use a separate pipette for leucocyte counting, and a fluid which by laking the red blood-corpuscles leaves the leucocytes the only cells in the field. The best fluid is dilute acetic acid, from 0.3 to 1 per cent. A 0.3 per cent. solution is hardly strong enough, for the red blood-cells are not entirely laked, hence groups of shadows are seen which are very confusing. This does not occur if a stronger acetic acid is used. Our method is as follows: We give each student three bottles, one of glacial acetic acid, the second of distilled water, and this should be frequently renewed, and a third, a small bottle of about 30 cc. capacity with a wide neck and a label stating how many drops of the glacial acetic acid are to be added to the bottle filled with distilled water to the line of the neck to get a dilution just under 1 per cent. This mixture is made up fresh each day. A dilute acetic acid much older than this should never be used, for yeast-cells grow which if single will resemble mononuclear leucocytes. In case there are many and in chains they are at once recognized, but we know of too many instances in which a count slightly too large was reported because a few were present. The pipette used may be the same as that used in the red blood-count but the blood should be drawn to the 1 point, thus giving a dilution of 1 : 100. Better pipettes are those (see Fig. 94) which give a dilution of from 1 : 10 to 1 : 40, since the greater the number of leucocytes counted the smaller is the error. We do not agree that it is "not at all difficult" to use these big pipettes; they require more practice than the others. Their bore is so large that the blood easily drips out; it is difficult to wash the blood entirely into the bulb by means of the acetic acid; and in shaking it it is easy to shake the leucocytes into the fluid filling the tube. To reduce these errors as much as possible, while the blood is drawn and while the acetic acid is aspirated into it, the pipette should be held almost horizontal; a wide-mouthed bottle of acetic acid should therefore be used, which allows of an almost horizontal position of the pipette. The acetic acid should be sucked in rapidly, that the stream may wash the tube well. The pipette is shaken in all directions except in that of the long axis of the tube. In this case also the specimen should be first observed with the low power to make sure that the distribution is even.

If the counting slide has the Thoma ruling, hence but 1 sq. mm. for use, this area from at least eight different drops should be counted. The Ewing, Zappert, or Türk rulings are to be preferred, since 9 sq. mm. from each drop can be counted. This should be repeated with three different drops. At least one hundred leucocytes should be actually counted, and more if possible. If the acetic acid be of the proper strength and fresh, and the pipette clean, all objects seen may be counted. The number of cells found divided by the number of units

counted and multiplied by 10, and this by the dilution, will give the number of leucocytes in 1 cmm. of undiluted blood.

Beware of nucleated reds, since their nuclei are similar to small mononuclears. The hour of the count should always be stated, and also whether a short time before the count the patient had partaken of a heavy proteid meal.

The error in leucocyte counting is usually at least 5 per cent. If a large number of leucocytes are counted, as was done by Reinert, the error will be about 3.5 per cent. We are sure that the error in the ordinary blood-count made by the busy ward man is nearer 10 per cent. We wish to emphasize this fact, for too often the clinical man who does not himself count blood draws from the counts made by his assistants conclusions concerning a rise or fall of leucocytes based on differences which fall within the limits of accuracy of the method as they apply it. We hear, for instance, of a rise of leucocytes from 10,000 to 11,000 or from 20,000 to 22,000 per cubic centimetre and so on, and are confident that the blood is not nearly so much to blame for variations of this amount as was the worker. A careful man will by repeated controls of his counts make sure that his technique contains no error over 5 per cent. This can be done by filling at the same time several pipettes, which are then separately counted, or better by occasionally inviting another, in whose work he has confidence, to make a series of parallel counts with him. In control work the blood should be taken at the same time and from the same incision, for one can obtain curious results by taking his blood from different parts of the body if he does not observe due precaution to avoid the ear on which the patient has been lying or the hand which has been in a hanging position for some time.

**Blood Smears.**—For satisfactory stained specimens the first necessity is to get good smears, thin, with the cells well spread and only few overlapping. The method we employ is the Ehrlich, using two cover-glasses. The cover-glasses, three-quarters of an inch square and of the thinnest glass and best quality, are thoroughly cleaned in alcohol and ether (see p. 448). and then dried. One cover-glass is held on one edge by the crossed-bladed forceps. The other cover-glass is placed in a convenient position to be quickly picked up by a pair of ordinary pinch forceps. A small drop of blood about the size of a small bead (about 1.5 mm. in diameter) is picked up on the last-mentioned cover-glass which is then at once dropped onto the other cover-glass. If these covers have been properly cleaned the blood will spread out rapidly from the weight of the cover-glass alone, and without the assistance of any pressure, which should be carefully avoided. Just as the spreading of the film is about to stop, but before it does, the two covers are pulled apart in a line parallel to their plane by a steady

but quick motion (see Fig. 98). With a little practice one will become quite skilful. Beginners find it easier to hold the free cover-glass in their fingers, but most workers disapprove of this, for the moisture from the fingers does affect the specimens to a slight degree. After one has had considerable experience, one hundred or more smears, a number which must often be prepared for class work, can be made much more quickly if two pairs of forceps are used. As soon as the covers are drawn apart the smears are waved in the air until dry; they should not be warmed over a flame. They then remain spread out on a sheet of paper from fifteen to thirty minutes to become still drier, but must be watched, for flies work havoc with them by sucking up the hæmoglobin, making large holes in the specimens. For some stains the smear is not allowed to dry, but is dropped at once into the fixing fluid. Smears should be guarded from dust and moisture.

Some prefer to make the blood specimens on slides. A large drop of blood is placed on a slide and is at once spread by drawing the drop along the slide with the edge of another slide, or, better, with the ground edge of a glass spreader, or better still, with the edge of a strip of paper; while a fourth method is to spread the drop by drawing a needle flat across the slide. In this

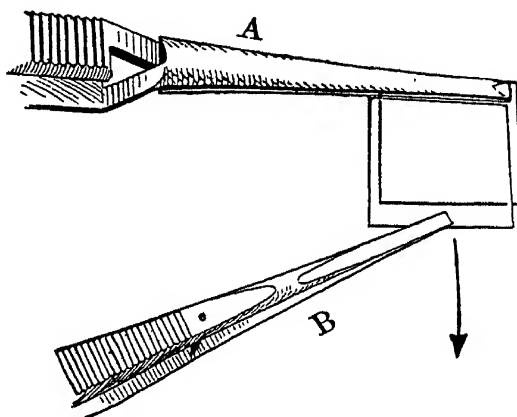


FIG. 98.—Method of making cover-glass preparations.

last way beautiful specimens may be obtained. The use of slides has two advantages—no cover-glasses need be used, and a much larger blood surface is obtained for study. On the other hand, only a few smears can be made at a time; more blood must be used; a large number of slides cannot be handled as easily as the same number of covers; and as a rule the spreads are not even. There is great need of getting uniformly spread specimens. Their edges should be thin, as the distribution of cells is never even, the leucocytes being found especially at the edges of the smear, and the platelets at the first point touched by the drop. Several smears have been sent us as illustrations of extreme leucopenia. In one case it was claimed that not a single leucocyte could be found, but they were found in abundance by those who know the tricks such specimens play. When the technique is slow masses of leucocytes are found on the area first covered by the drop, and here one gets the mistaken notion of an extreme leucocytosis. But even when two cover-glasses are used the picture is not just the same on both.

It is of the greatest importance to obtain good specimens. With the most careful technique some of the most interesting cells are nearly always ruined. We refer to the macrophages and to the very large mononuclear cells which can be found in sections of drops of blood hardened *en masse*, and in fresh specimens.

The smears thus dried in the air may be kept some time. One obtains better results with Ehrlich's stain by heating after three or four days than on the first day, while in the case of the many methylene blue-eosin stains now in vogue it is much better to stain at once, even before they are air-dry.

**Fixing Methods.**—The fixing method used will depend upon the stain which it is intended to employ. Among the various chemical methods are:

(1) NIKIFOROFF'S, consisting of absolute alcohol and ether equal parts; the specimen is to be immersed for from a half to two hours. This method is particularly good for malarial specimens and for the degenerations of the red blood-cells.

(2) ABSOLUTE ALCOHOL for five minutes, or, if in a hurry, boiling absolute alcohol one minute.

(3) FLEMMING'S SOLUTION, particularly good for the study of the chromatin of nuclei, consists of chromic acid, 1 per cent., 15 parts; osmic acid, 2 per cent., 4 parts; glacial acetic acid, 1 part. The blood specimens, as soon as made and before they could possibly dry in the air, are plunged immediately into this fluid and left for ten minutes. They are then washed in running water for about ten minutes and dried.

(4) VAPORS OF FORMALDEHYDE.—The specimen is put under a bell-jar together with a few drops of formaldehyde, and is exposed thus to its fumes for about five minutes.

(5) The FUTCHER-LAZEAR method employs 0.25 per cent. formalin in 95 per cent. alcohol for one minute. To 10 cc. of 95 per cent. alcohol are added 4 to 5 drops of 10 per cent. formalin (*i.e.*, the strong formalin of commerce, 40 per cent., diluted with three volumes of water). This is always made fresh. The specimen is left for one minute, then rinsed in running water, and dried on filter paper.

(6) HEAT.—This method, the most difficult to use well, is the only method which gives satisfactory results with the very important Ehrlich triple stain. The best apparatus is that originally introduced by Ehrlich,—a large triangular copper plate, not polished, with a gas-burner under the point. This is allowed to heat until at a constant temperature, and then the boiling point determined by dropping water at different distances from the flame until the point is reached where it just boils. The cover-glass is placed blood-side up on the copper plate inside of the boiling point, that is, toward the flame,

with its outer margin about three-quarters of an inch from the boiling-point, that is, at a point where the temperature is from  $110^{\circ}$  to  $115^{\circ}$  C. Some prefer to use drops of toluol or xylol instead of water. Where these just boil is the point on the plate where the temperature is from  $110^{\circ}$  to  $115^{\circ}$  C. By heating the smears at this temperature from an hour and a half to two hours very good specimens may be obtained. It is best to place the specimens first at a cooler point, and then to move them gradually up to the desired point, and after they have remained there from one and a half to two hours to let them cool slowly, as too rapid changes of temperature may shrink or split the cells. The length of time during which specimens should be heated depends on two things, one of which is the age of the specimen. In case the blood is heated on the day when the specimen is made, two hours will be hardly long enough. The trained eye can usually distinguish specimens which were heated while very fresh by certain undesirable characteristics which they show. Specimens a week old generally require an hour and a half of heating, and older specimens usually less than an hour. The length of time during which the specimen should be heated depends also on the patient's disease. Normal blood requires the longest heating. Blood from a patient with splenomyelogenous leukaemia is usually ruined if left on the bar more than one hour, and to heat blood from a patient with pernicious anæmia a few minutes too long will often spoil it. Our method is to place four cover-glasses on the bar at the point described above, and then to remove the first in one hour and the others later at intervals of about twenty minutes. One of these is almost certain to stain satisfactorily, and all the other smears are heated for the same time as this satisfactory one. Others keep the blood specimen at the boiling-point, but with the blood side down, about fifteen minutes.

The thermostat is a very convenient instrument to use, but one must be sure that its thermometer measures the temperature of the plate on which the cover-glasses rest, rather than that of the air above it. Various ovens are sold which give a very constant temperature as they are heated by fluids whose boiling-point is the temperature desired. Among these may be mentioned the Viktor Meyer, and lately the Ehrlich ovens. In these ovens xylol or toluol is used.

Others prefer to fix the blood by running the cover-glass, and especially the slide if the smear is on that, through the flame two or three times, just as smears of bacteria are fixed. In this way splendid specimens are often obtained. Still others prefer the "spheroidal-point method." They determine on the copper bar that point where the drop of water just rolls off without boiling, that is,

where the temperature is from  $140^{\circ}$  to  $150^{\circ}$  C. At this point the smears lie face up, for from thirty seconds to two minutes.

In the case of the Ehrlich stain the success in staining will depend on the heating; if the specimen appears over-stained it was under-heated; if unstained, it was overheated. The red blood-cells are a good index; they must show no fuchsin tint whatever, nor again a lemon-yellow. The amount of stain which the plasma will take also depends on the time heated; there should be no halo around the corpuscles, under-heating bringing out Deetjen's "cell membrane."

WOOD ALCOHOL is, however, the fixative preferred by most since it can be mixed with the stain, hence the fixing requires no extra work.

In the choice of a method the subject studied must be taken into consideration. For the granulations heat is the best method, since it allows the neutrophile granules to take the specific stain; osmic acid 2 per cent. at a temperature of  $37^{\circ}$ , the specimens before they are air-dried being exposed to these vapors; or chromic acid 1 per cent., the specimen fixed for from two to ten seconds and then washed in distilled water at once for from two to ten seconds, may also be used. For the study of the nuclei, Flemming's fluid or the Nikiforoff method is best; for protoplasm, chromic acid, osmic acid, or alcohol and ether.

**Stains.**—Stains have been classified by Ehrlich as acid, basic, and neutral. These words do not refer to their chemical reaction, but to the portion of the compound which does the staining. The classical illustration of this is the following: ammonium picrate is an acid stain, since it is a picric acid which gives the color; rosanilin acetate is a basic stain, since it is the basic element which is efficient; as an illustration of a neutral stain rosanilin picrate would serve, since both the basic and the acid parts would stain. As a matter of fact the neutral stains are all of them mixtures of one or more stains, and it is very hard to state just how the compound arises and what it is.

Among the basic stains may be mentioned methyl green, methylene blue, fuchsin, methyl violet, Bismarck brown, and saffranin.

Among the acid, eosin, aurantia, the salts of picric acid, indulin, acid fuchsin, orange G, and a long list of others.

Neutral stains arise in mixtures of the above. For instance, of acid fuchsin and methyl green; of methylene blue and eosin.

**Methods.**—(1) Fitcher and Lazear recommend a SATURATED SOLUTION OF THIONIN IN 50 PER CENT. ALCOHOL, to 20 cc. of which 100 cc. of 2 per cent. carbolic acid are added. This stain is allowed to stand for some time. The specimens fixed by the alcohol-formalin method (see p. 476) are stained in this for from ten to fifteen seconds. This was particularly valuable for malaria specimens, the hyalines showing as reddish-violet ring-like bodies.

(2) **HÆMATOXYLIN EOSIN.**—This stain is not used nearly as much as it deserves, since there is no better way of bringing out the nuclei.

**MAYER'S SOLUTION.**—Hæmatoxylin, 1 gm.; alcohol, 100 cc.; while cool, 50 gms. of alum are added, and then 1000 cc. of boiling distilled water. A few crystals of thymol are then added, the whole cooled, and filtered. It is to be kept in the dark. Only experience will tell how long the specimens are to be stained. They are afterwards washed rapidly in water. The nuclei will alone take the color. Eosin, 0.5 per cent. aqueous solution, may then be added until the red blood-cells are just rose-red. The specimen is then washed in water, dried, and mounted. The protoplasm and the nuclei are beautifully stained, but the granules not so well.

**Ehrlich's mixture:** Eosin (cryst.), 0.5 gm.; hæmatoxylin, 2 gms.; absolute alcohol, distilled water, glycerin, āā 100 gms.; glacial acetic acid, 10 gms.; alum, in excess. This is allowed to stand for several weeks. The specimens are stained for from one-half to two hours.

(3) **PAPPENHEIM'S SOLUTION OF PYRONIN AND METHYL GREEN** is composed of saturated aqueous solution of methyl green, 3 to 4 parts, and saturated aqueous solution of pyronin, 1 to 1½ parts. This stain, which may be used as a routine bacterial stain, is useful also in blood work, for distinguishing the nucleus of the erythroblast from its basophilic granules. The nucleus and all nuclear fragments stain a beautiful blue, the basophilic granules a brilliant red (Morris). The blood spreads should be fixed by heat (Ehrlich's method).

(4) **EHRLICH'S TRIPLE STAIN.**—The words Ehrlich's "triacid" and Ehrlich's "triple" stain are often wrongly used as synonyms. The triacid stain was a mixture of indulin, nigrosin, and aurantia, equal parts of the saturated solutions. This stain was to bring out the eosinophile granules. It is hard to make up, and is now very little used. By "triacid" is usually meant the following stain.

Ehrlich's triple stain is a mixture of the saturated aqueous solutions of methyl green 00, acid fuchsin, and orange G. (Grübler's stains are usually used.) These solutions are allowed to stand at least one week, and if longer give still better results.

The best formula for this stain is that published by Morris.\*

	cc.
Saturated aqueous solution of orange G .....	13.0
Saturated aqueous solution of acid fuchsin .....	7.0
Distilled water .....	15.0
Absolute alcohol .....	15.0
Saturated aqueous solution methyl green.....	17.5
Absolute alcohol .....	100
Glycerin .....	10.0

\* Jour. of A. M. A., Aug. 6, 1910, vol. 1v, p. 501.

These fluids are measured in the same graduated cylinder, which should not be rinsed. The receiving flask should be shaken vigorously after the addition of each of the constituents, and these are added in the order given in the formula. It is essential to add the methyl green, the second portion of alcohol, and the glycerin slowly and to shake the flask well after each addition. The mixture is ready for use immediately and does not deteriorate with age.

This stain seems to improve for a time by standing, but when old it is sure to spoil. The bottle containing it should never be shaken, and it should not be filtered. The drops to be used are removed on a glass rod from as near the centre of the bottle as possible, and the specimen is kept covered with them from three to twenty minutes. It is very difficult to stain too much and films suggesting an excess of staining are usually underheated. The smear is next washed in distilled water, quickly blotted, and mounted in balsam. It may be washed quickly in absolute alcohol, which brings out the granules more clearly, but which makes the nuclei paler. In a successful specimen the red blood-cells will be of a buff color without the slightest shade of red; the nuclei of the leucocytes will be of a dark green, those of the normoblasts almost black; the neutrophile granules will take a lilac stain, and the eosinophile granules a crimson. This is the only stain which gives a specific color to the neutrophile granules, and it is for this reason that it was introduced. It is inferior to other stains for protoplasm and nuclei, and does not in the least stain the Mastzell granulation. If one desires to get a good idea of the blood as a whole, other stains also should be used, preferably hæmatoxylin and eosin, or methylene-blue and eosin, etc.

The blood of some persons takes the Ehrlich stain poorly, while that of others takes it well. In certain diseases, particularly lymphatic leukæmia, it is almost impossible to get a good specimen with Ehrlich's stain because the basic element is so markedly lacking.

There seem to be individual peculiarities in bloods. Our students are required to stain their own blood until satisfactory smears are obtained, in order that they may learn how to judge of specimens. Many students will succeed the first time; some will make from 100 to 150 specimens from their own blood without getting one satisfactorily stained smear, and others will have no better success with these same bloods. Certain peculiarities of the staining qualities of cells are so marked that it is sometimes possible to tell whose blood is under the microscope, provided the observer has already studied that blood. In following the work of ninety students during one year, we were more than ever convinced on this point. The staining



qualities of bloods depend on certain other factors quite as much as on the fixing and staining technique.

The POLYCHROME METHYLENE BLUE-EOSIN STAINS are at present the favorites, since they are easy to use, contain the fixative, and give fairly satisfactory results. In the case of malaria they are the best stains to use; since it is only they which bring out the chromatin of the parasite. For blood smears they are satisfactory; the nuclei stain very well, also the Mastzell granulation, and the protoplasm. The eosinophile granulation can be easily recognized, and the neutrophile granules stain perhaps as well as is necessary, and may be recognized from their fine size and purplish tint. However, if one is studying granulations, he will not use this stain alone, nor, indeed, any stain containing methylene blue, which is very tricky. At least sixteen different methods<sup>8</sup> of making this stain have been reported, all of them modifications of the original Romanowski. The method which we use is that described by Hastings in the Johns Hopkins Hospital Bulletin, 1905, since it is one of the easiest to make up and so seldom fails.<sup>9</sup>

HASTINGS' STAIN.—The dry stains necessary are eosin, soluble in water, yellow (Grübler); and methylene blue (Ehrlich's rectif.) (Grübler).

Solution A = eosin 1 per cent. aqueous.

Solution B = alkaline methylene blue 1 per cent. aqueous.

Solution C = methylene blue 1 per cent. aqueous.

Solution A may be kept ready-made; solutions B and C must be made fresh.

To prepare B use warm 1 per cent. solution of dry powdered sodium carbonate. Add to it 1 per cent. of methylene blue powder and heat over a water-bath for fifteen minutes. Add 30 cc. of water for each 100 cc. of original fluid, and heat again fifteen minutes. Then pour off the solution from the residue, divide into two equal parts, and to one part add enough 12.5 per cent. acetic acid to faint acid reaction. This is best determined by placing a drop on blue litmus paper and taking as the end reaction the point at which the margin of the drop after absorption in the paper shows a faint pink. Then add the remaining unneutralized portion to this.

To mix the stain use distilled water, 1000 cc.; solution A, 100 cc.; solution B, 200 cc.; solution C, 70 to 80 cc. In adding solution C, put in 70 cc. at once, stir well, and if no precipitate is present add a cubic centimetre at a time until one appears. After the precipitate appears the stain is allowed to stand for half an hour, and then filtered through one filter. Forced filtration is usually necessary.

<sup>8</sup> Baumgarten, American Med., 1904, vol. vii. p. 14.

<sup>9</sup> See, also, Wright's Method, Jour. Med. Research, 1902, vol. ii. p. 138.

The dry residue is removed from the paper, powdered up, and may be kept in this form or dissolved in Merck's pure methyl alcohol. Seven- to nine-tenths of a gram of dry stain is usually obtained. Three-tenths of a gram dissolved in 100 cc. of alcohol gives the staining solution. In dissolving the stain it must be rubbed up with the alcohol in a mortar and pestle, as it is with difficulty soluble.

If more than nine-tenths of a gram of dry powder is obtained the resulting stain is useless. For each new lot of stain made up one must determine the relative proportions of stain and water to be used in staining and the relative lengths of time in which to let the pure and diluted stain act. Usually 2 drops of stain for one minute on the smear and then with 4 drops of water to it for four minutes gives the best result. For uniformity in the size of drops a dropper should be used. The two drops of undiluted stain for one minute fixes the specimen, which, after the addition of the water, receives its differential stain.

All of these methylene blue-eosin stains require experiment, since different mixtures by the same method require slight variations in their use, learned only by trial. The reason for accuracy of dilution is to prevent a fine black precipitate, which detracts much from the beauty of the specimen. This precipitate may be removed by slight decolorization in 95 per cent. alcohol. If too much decolorized, it is the red chromatin of the malarial parasite which suffers the first. Only the purest methyl alcohol should be used; use distilled water to wash the specimen, since tap-water sometimes ruins it.

*Wilson's Stain.*—Make a 1 per cent. solution of methylene-blue in a 0.5 per cent. aqueous solution of sodium carbonate and add at least 0.5 per cent. of freshly precipitated silver oxide. (To prepare the silver oxide, dissolve 2 gms. of  $\text{AgNO}_3$  in 15 cc. of distilled water and add about 260 cc. of milk of lime. Shake well and set aside. Decant the supernatant fluid. Collect the precipitate on a filter, wash it with about 20 cc. of distilled water, dry it at a temperature not exceeding  $100^\circ \text{C.}$ , and preserve it in a tightly-stoppered dark bottle.) Boil the methylene-blue solution and at the end of 20 minutes remove one-third of it. After 20 minutes' more boiling remove one-half of the liquid and boil the remainder 20 minutes. Combine the three portions of fluid and make the mixture equal to the original volume with distilled water, discarding the precipitate which sticks to the bottom of the evaporating dish. Mix the methylene-blue solution with an equal volume of a 0.5 per cent. aqueous solution of eosin and allow it to stand one hour. Collect the precipitate on a "hard" filter paper, wash it to remove free bases, with distilled water or, preferably, with 0.85 per cent. sodium chloride, dry it, and preserve it in a dark glass bottle.

To prepare the staining mixture, 400 mg. of the powdered stain are dissolved in 100 cc. of absolute methyl alcohol. Since the powder is only slightly soluble, solution is facilitated by rubbing in a mortar. The stain should be kept in a tightly-stoppered bottle to prevent evaporation of the methyl alcohol. A dark bottle is advantageous.

The cheaper grades of methylene-blue are said to make satisfactory stains.

If the stain used is one in which the cover-glasses are to be completely immersed, much time may be saved by using the holder which Pepper\* has invented. This allows 55 slips to be stained simultaneously in 35 cc. of the staining fluid.

For basophile granules the methylene blue stains, carbol thionin, or dahlia may be used.

Another carbol thionin mixture is thionin, 0.3 gm.; absolute alcohol, 10 cc.; carbolic acid, 1 per cent., 100 cc. The fixed smear is stained two minutes, washed in water, and dried.

Ehrlich's dahlia stain consists of distilled water, 100 cc.; saturated alcoholic (absolute) dahlia solution, 50 cc.; then, on clearing, 10 to 12.5 cc. of glacial acetic acid. The specimens (heated or fixed by alcohol, etc.) are stained for from five to ten minutes.

#### SPECIFIC GRAVITY OF BLOOD

(1) **Gravimetric Method.** (a) **PYCNO-METER.**—This method is certainly the most accurate, but requires considerable blood (at least 5 cc. for an accurate estimation) and a very accurate chemical balance.

(b) **SCHMALZ'S TUBES.**—This method is less accurate than the above, of which it is a modification. A tube is used which holds about 0.1 cc. of blood. It is about 12 cm. long and 1.5 mm. wide, and slightly constricted at the ends to prevent any loss of blood. The tube, well dried, is weighed on a chemical balance which is accurate to at least 0.1 mg. It is next filled with distilled water and weighed again. Then it is cleaned thoroughly, dried, filled with the blood, and weighed once more. If  $c$  equals the weight of the tube,  $c'$  the weight of the tube filled with water,  $c''$  the weight of the tube filled with blood, then  $\frac{c'' - c}{c' - c} = \text{sp. gr.}$  This method, while fairly accurate, requires considerable skill.

(2) **Aræmetrical Methods.**—By these methods the blood is dropped into fluids which will not mix with it, and the specific gravity of each of which is known. If the drop of blood rises in the fluid, the specific gravity of blood is less than that of the fluid; if the blood sinks, its specific gravity is greater than that of the fluid. The Roy

\* Jour. of A. M. A., Jan. 11, 1908, vol. 1, p. 122.

method requires the use of a series of bottles containing fluids which vary in specific gravity and into which drops of blood are introduced until a fluid is found in which the drop neither rises nor sinks.

In the Hammerschlag Method a mixture of benzol and chloroform is used, and this mixture is modified until it has the right specific gravity. A glass cylinder, perfectly clean and dry (else the blood will cling to the side of the glass), is filled with the mixture mentioned above, of which the specific gravity is about 1058. A drop of blood is then introduced, best through a capillary tube bent at the end at right angles, so that the drop may be blown into the fluid without any upward or downward motion. If the drop rises, benzol is added; if it sinks, chloroform. After each addition the fluid must be well stirred. The mixture evaporates, its specific gravity changes and there is some exchange between the blood and the fluid. For these reasons it is important to work very rapidly, to confirm the final result by a fresh drop of blood, and to test the specific gravity of the mixture without delay. The drop of blood may then be removed by filtering the mixture through linen before its specific gravity is tested. Care must be taken that no bubble of air sticks to the drop. Slight differences in the temperature of the mixture make considerable difference in the result. (So great is the influence of temperature on the specific gravity of the mixture that Langlois varies, not the proportions of benzol and chloroform in the mixture, but its temperature. When the drop no longer rises or sinks he reads the temperature of the mixture and from this reckons its specific gravity.)

The specific gravity of the blood serum may be tested as follows: a tube is filled with blood, both its ends are sealed and it is allowed to stand upright until the serum has separated well from the clot. The tube is then broken, and a drop of the serum tested in the same way as was the blood. That of the plasma may be tested by filling with blood a glass tube which has been washed out with 3 per cent. oxalic acid to prevent clotting. This is then sealed, the cells allowed to sediment, and the supernatant plasma examined. Hammerschlag considers that this small amount of oxalic acid will not affect the result.

In conclusion, the Hammerschlag method looks easy and is simple, and yet the possibilities of error from a bubble of air in a drop, the evaporation of the mixture, imperfect mixing of the two component fluids, and the change in specific gravity of the blood from contact with this mixture are great.

The specific gravity of normal blood has been variously stated. Ehrlich considers that it varies normally from 1058 to 1062, the average for man being 1059, for woman, 1056. The figures given by Piper are, for man, 1055; for woman, 1053; for children, 1051.

Landois states the average is 1054, the normal limits being 1045 to 1075. Lloyd Jones places the limits at 1036 to 1068, and Hammerschlag from 1056 to 1063. It is seen from the above figures that the specific gravity of the blood of a woman is slightly less than that of a man. At birth Lloyd Jones found it 1066. It drops, reaching a minimum of 1048 to 1050 in the second year, and then rises to a maximum, which obtains between the ages of thirty-five and forty-five, of even 1058; after the menopause the average is 1054. The rise in adult life may continue to even 1066. Diet has little effect. Menstruation, Schmalz says, is followed by a slight increase. Daily variations are noted by Schmalz, the maximum between 7 and 8 A.M. being 1060.7, and from 11 A.M. to 8 P.M. 1058.8. The specific gravity for the serum and the plasma is about the same; from 1029 to 1032, an average of 1030. The specific gravity of the plasma, while much more uniform than that of the total blood, nevertheless is diminished in dropsical condition.

Using the Hammerschlag method, twenty-three of our students, normal men, ages between twenty and twenty-five, found their blood to vary from 1051 to 1065. In the case of sixteen of the twenty-three it was from 1057 to 1061; the mean of all was 1058.

In pathological conditions the specific gravity of the blood may vary from 1025 to 1068, in most cases running parallel to the hæmoglobin. It is reduced in all anæmias, especially in chlorosis. It is reduced in many cachexias, in which case the change is in the plasma, for the hæmoglobin may be practically normal. It is increased in fevers from 1057 to 1063, in cyanosis, in obstructive jaundice.

Until the introduction of the Miescher hæmoglobinometer the specific gravity was the best method for the determination of the hæmoglobin, especially in some anæmias as chlorosis, in which cases the change in specific gravity is due almost entirely to the variations in the amount of hæmoglobin. In cases with hydræmia, however, this rule does not hold, since there the loss is also due to changes in the water of the plasma.

It has been found that 10 per cent. hæmoglobin is equivalent to 4.46 per mille specific gravity, but with the hæmoglobin the same specific gravity can vary even 13.5 per mille. If the color index is changed, the element of the stroma enters even from 4 to 5 per mille, the absolute amount of hæmoglobin being the same. In leukæmia the hæmoglobin thus estimated is too high, while in pernicious anæmia about 2 per cent. too low. In cases of hydræmia the method cannot be used at all; for instance, in cases with dropsy, anæmia from malnutrition, post-hemorrhagic anæmia, and circulatory disturbances, in which the plasma is considerably affected. In fact, about the only condition

in which it has been used with good advantage is in chlorosis. The specific gravity of the plasma is fairly constant, the change in the water affecting especially the red blood-cells. This is true even in severe blood diseases, as, for instance, in pernicious anæmia. Since the Miescher instrument has come into use there is no longer very much value in this method for hæmoglobin determinations.

**Dried Residue. Hygrometry.**—A weighing glass with a ground-glass stopper is first carefully dried and weighed. A little blood is then introduced, the cover is put on, and it is weighed again. It is then dried for twenty-four hours, or to constant weight, at a temperature from 65° to 70° C., and then its weight determined. The solids of the blood in the case of the normal man average about 21.6 per cent.; for the woman, 19.8 per cent. The figures of Askanaazy are, for man, from 20.35 to 22.89; average, 21.92 per cent.; woman, from 19.58 to 21.46; average, 20.53 per cent.

For the study of anæmias it was hoped that this would throw important light upon the condition of the blood, since it was found to run not parallel to the specific gravity nor to the count of the red blood-cells, nor to the hæmoglobin, and it thus seemed an independent element. Its value has, however, not proved as great as was hoped.

**Sedimentation of the Blood.**—The estimation of the volume of the red blood-corpuscles would it was hoped dispense with the hard and tedious process of blood-counting, since men said, after all it is not so much the number of the red blood-cells as the volume of hæmoglobin-containing protoplasm which is important. The volume of the red blood-cells may be determined by the centrifuge method with the hæmatocrit and undiluted blood (see page 471), or the centrifuge, the blood diluted with an equal volume of potassium bichromate 2.5 per cent. or Müller's fluid. The value of the results by this method is hardly great, since the compressibility of the red blood-cells seems to vary in different conditions.

The spontaneous sedimentation of the red blood-cells is recognized as a more accurate method than the centrifuge.

Marcano's method: Sodium sulphate solution, sp. gr. 1020, 100 cc.; sodium chloride, 1 gm.; formalin, 3 cc.

In a special pipette the blood is diluted four times with the above-mentioned fluid, and then blown into a graduated conical glass and allowed to stand twenty-four hours. The volume of the red blood-cells may then be read directly.

The normal volume is 50 per cent. In chlorosis it often runs as low as 20 per cent., while in pernicious anæmia even 9 per cent., in general depending on the count, but the determination of which it cannot replace.

**Coagulation.**—On few subjects in hæmatology has so accurate, careful scientific work been done as on the coagulation of the blood, and the results are of unquestionable value in our clinical work. In this chapter we shall quote freely from Hinman and Sladen.\* The subject is a difficult one. There are at least three forms of coagulation to consider—that in an open wound, thrombus formation in a closed vessel, and coagulation within our laboratory instruments. These processes are very different, and “we cannot bring the appearance of coagulation in the living vessel into direct parallelism with coagulation of blood as ordinarily understood” (Welch), nor can we reproduce the conditions under which either occurs. Thrombosis is a very common complication, in typhoid fever, anæmia, and cachexia, and yet the amount of fibrin demonstrable in the blood in these conditions is quite low; on the other hand, the blood in pneumonia and in acute articular rheumatism is very rich in fibrin, and yet, in these conditions, thrombosis is of rare occurrence. Again, coagulation in the wound is not a uniform process. More depends on the nature of the vessels cut, on the tissue through which the blood escapes, and on the environment it incurs, than on the size of the vessels cut. It is scarcely possible for a man to die from hemorrhage which would follow cross-section of a radial artery, large as that is, while the fatal intestinal hemorrhages in typhoid fever are from vessels so small that they cannot be found without a microscope. The character of the vessel's wall, the opportunity for it to contract, the character of the tissue of the Peyer's Patch, and possibly the intestinal contents,—all these may conspire to make the intestinal hemorrhage much more serious than the arterial. Then, too, the rapidity of coagulation of blood in a tube or glass chamber depends on many factors, few of which are understood. For instance, the longer the blood is in contact with the cut tissues, the more rapidly will it clot; blood from a deep wound will clot even three minutes more slowly than will that from a superficial wound; each drop of blood flowing from a wound will clot more quickly than will the preceding drops, so that if several are allowed to flow the difference in coagulation time between the first and last drop may be almost ten minutes; the pressure made on the flesh near the cut to encourage the flow of blood, the amount of blood used, the material composing the receptacle in which it clots, this receptacle's cleanliness and temperature, the temperature of the air, and the opportunity there is for evaporation,—all these modify the rapidity of coagulation. The above considerations show the need there is of as uniform technic as possible.

Even if the technic were as uniform as possible, it would still be

\* The Johns Hopkins Bull., July, 1907, vol. xviii, p. 207.

true that blood removed at the same time from different parts of the body will clot with different degrees of rapidity; that the diet and especially the medicines are factors to be considered; and that the coagulation time differs appreciably at different times of the day. (The longest time is soon after breakfast, when the blood of normal men sometimes clots in from 12 to 17 minutes, a slowness which at any other time of day would be distinctly pathological. The most rapid coagulation occurs about four o'clock in the afternoon.)

While we have not yet been able by our clinical observation of the coagulation of blood to gain much data, if any, which gives us a better understanding of internal coagulation, as in thrombosis, the clotting of effusions, etc., yet the observations made on cases with hemorrhagic diatheses, as the purpuras, hæmophilia, and the anglo-neurotic oedemas, are of interest. Surgeons consider the determination of coagulation time of great importance, and now few of them would operate on a jaundiced patient unless this determination had been made in advance.

A few doses of calcium chloride or lactate will shorten the coagulation time considerably, but if this medication be continued the time will be lengthened.

A normal coagulation time has chiefly negative value. If the time is clearly slower than the limits of normal, it may mean danger, although the danger indicated may prove a false alarm. Hinman and Sladen give the following illustration of this point: The coagulation time in a case of hæmophilia was  $16\frac{1}{2}$  minutes, that is, it was distinctly prolonged. The prick in the ear by which the drop was obtained closed at once without further bleeding. On the same day the same ear was pricked a second time. The drop obtained clotted in  $18\frac{1}{2}$  minutes, but this prick bled for twelve hours. If the coagulation time is short, it is almost always easy to stop bleeding through a skin wound.

*Estimation of Coagulation Time.*—"Coagulation time" is not the time it takes blood to clot in a wound, for we have no means of measuring that. It is the period which elapses between the appearing in the wound of the drop of blood to be tested and the first moment when there is distinct evidence of fibrin formation in the blood in the instrument. One does not measure the time from the moment the blood is transferred from the wound to the instrument. For comparable work, all determinations should be made approximately at the same time of day; the blood should always be obtained from the same part of the body; since one cannot expect to get a uniform flow of blood he should at least get a free flow; the prick should not be made in the seat of an active or a passive congestion (for tissue



lymph and carbon dioxide both hasten coagulation); the second or third drop which wells out should be used, not the first, and not the later drops; the temperature of the room in which the observation is made should not be unusually warm or cold, although one need not try to control the temperature beyond preventing extremes; and anything which tends to increase the drying of the blood, as a draught of air, should be avoided.

The method used to determine the coagulation time should be one which guarantees the uniformity of size of the drops used, and permit the observer to prove objectively the presence of fibrin at the time when he considers the drop clotted. In some cases the drop of blood dries and becomes apparently clotted before a demonstrable amount of fibrin has formed.

Among the older methods is Hayem's, who received the blood into a graduated cylinder and considered it coagulated when the cylinder could be tilted without the blood mass changing shape. Another way was to receive a large drop on a clean slide and test its consistency from time to time with a needle. Others put on a cover-glass and watched for the appearance of fibrin with the microscope. The above have been discarded, since the results were never uniform.

**VIERORDT'S METHOD.**—This method has simplicity to recommend it. A white horse-hair 10 cm. long is boiled in alcohol and ether. A capillary tube 5 cm. long and of 1 mm. bore is thoroughly washed and dried also in alcohol and ether. A drop of blood giving a column about 5 mm. long is received into the tube and the white horse-hair run through it. Each minute the hair is pulled slightly through the drop. The first appearance of coagulation is shown by a slight reddish stain on the hair, which after the blood is well coagulated will again appear clean. It is of greatest importance that that part of the horse-hair which is to come into contact with the blood should not have been touched with the fingers. The amount of blood should be exactly the same each time, since the coagulation time depends directly upon the amount of blood.

All results should be confirmed by a second determination.

If no better apparatus is at hand the simple drop method may have a little value. A drop of blood constant in size is placed on a clean slide. From time to time this slide is tilted. One notes the time when the drop has elasticity of form, that is, has ceased to act as a fluid.

The method now considered the best is that of **RUSSELL AND BRODIE**. A microscope is necessary. The apparatus consists of a moist chamber with a glass bottom which can be placed upon the stage of the microscope, while the upper surface is a truncated cone of glass projecting downward into the moist chamber. The lower surface of this is of a definite size (about 4 mm. in diameter), and on it is placed a drop of blood, care being taken that the drop only just covers the surface, hence is always of the same size. The glass is then quickly fitted into the moist chamber. Through the side of this chamber projects a fine tube, through which, by means of a bulb, a gentle stream of air can be directed against the blood. With the low power

of the microscope the cells are then watched as thus agitated until they are seen to move in clumps.

This method is the most accurate yet devised. The original apparatus of Russell and Brodie<sup>11</sup> has been modified recently, a much cheaper one devised by Pratt, in which the glass cone is dispensed with, and a still better one by Boggs. The Boggs apparatus has the

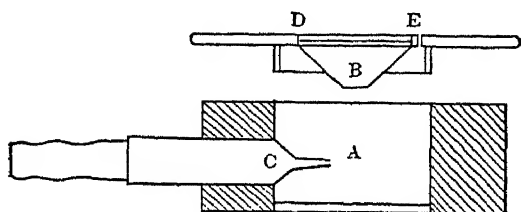


FIG. 99.—Coagulometer of Russell and Brodie as modified by Boggs. A, moist chamber; B, cone of glass the lower surface of which holds the drop of blood, C, side tube; D and E, cover-glass; at E, a pinhole.

advantage of a metal tube and the improved glass cone, although the peripheral jacket, in which water of a certain temperature can be circulated, is not present, nor is this very necessary. (See Fig. 99.)

As little blowing and at as long intervals as possible should be done.

The corpuscles will at first move freely and independently of one another (see Fig. 100, A), then in clumps on the periphery, B. As the process of coagulation continues, the masses of corpuscles will no longer move in the drop, but the drop changes shape en masse, the corpuscles showing first an elastic concentric motion, C, and finally an elastic radial motion, D; that is, the current of air will cause the masses of corpuscles to move toward the

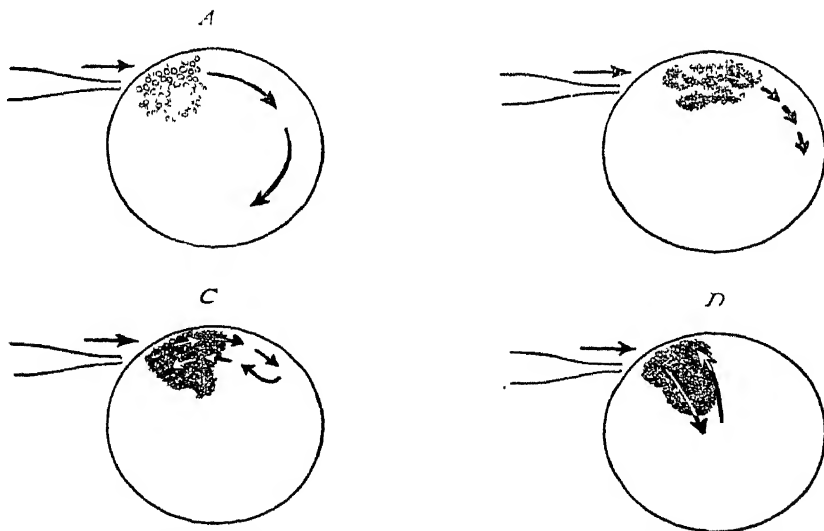


FIG. 100.—Diagram to illustrate the movement of the cells during coagulation.

centre, and to quickly spring back to their original position when the current of air ceases. This is taken as the final point, since only now can a clot be demonstrated if the disk be quickly removed and the drop be touched to a piece of filter paper. All clots should be confirmed in this way. Sometimes a "vicious circle" is set up in the drop, which clots

<sup>11</sup> Journ. Phys., May 12, 1897.

everywhere but one point where the blood remains fluid. Such a drop should be discarded and another attempt made. It is due to too hard blowing.

Successive records at intervals of 5 to 10 minutes should not vary over 30 to 45 seconds.

MILLIAN'S METHOD is a modification of Hayem's. This method which is considerably in vogue among the French is to place a drop of blood on a clean glass slide, to cover it by a crystallizing dish to prevent very much evaporation, then at stated intervals to tilt the slide, and from the change in shape of the drop of blood can be determined the coagulation point. By using this method most remarkable results have been obtained, the coagulation time extending even into hours. The method has been tested under Dr. Boggs's direction in this clinic by Messrs. Hinman and Sladen, who have found that very much depends on the size of the drop of blood and the evaporation.

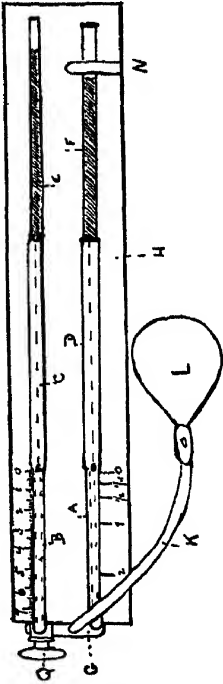
The coagulation time to be considered normal will depend not alone on the instrument, but on what is considered the end-point. Using the Boggs instrument Messrs. Hinman and Sladen found it to vary from three to eight minutes, an average of five minutes and six seconds, a longer time than some others, since they chose a later point as the end. (Brodie and Russell, three and a half minutes; Murphy and Gould, three minutes, eleven seconds; Pratt, four to five minutes.) Above nine minutes means delay in coagulation.

So many methods of such varying value have been used that it is difficult to put in order the findings concerning the coagulation. All are, however, unanimous concerning the following point: In the hemorrhagic diatheses the coagulation time of the blood is immensely increased. In some cases of hæmophilia it requires fifty minutes, while in certain of the purpuras from ten to fifteen or more. In long-standing jaundice the coagulation time is increased, a point which interests surgeons, and in this clinic in cases of jaundice with delayed coagulation an operation is never performed until it has been decreased to about five minutes by a long course of calcium chloride. The coagulation time is diminished in stasis due to any cause, after repeated hemorrhages, transfusion, hunger, by calcium chloride, and by carbon dioxide. In this clinic the gelatin injection method for the cure of aneurism has been given a fair trial and was finally abandoned, and further work throws considerable doubt on the value of the method. In this connection it has been shown that the gelatin of commerce contains considerable calcium, and that if decalcified gelatin be used the results are quite different.

**Fibrin Diagnosis.**—If very thick smears of blood be made, the fibrin may be seen to radiate in strands through the specimen, usually from masses of platelets. These smears are allowed to stand for hours under the bell-jar. If desired they may then be washed by a gentle stream of water which will remove the hæmoglobin, and the fibrin

left may be stained with fuchsin and the specimen mounted. When examined fresh the specimen should be closed with vaseline to prevent evaporation. Those diseases in which most fibrin is seen are pneumonia and acute articular rheumatism. In the former case it is suggested as a differential point against tuberculous pneumonia.

*The Viscosity of the Blood.*—The best instrument in use is Hess's viscosimeter. On a base of opaque glass H (see drawing) are fastened two graduated glass tubes, A and B, which are connected at one end by a third tube, G, while this cross tube in turn connects, through a branch, with a rubber balloon, L. At their other end the two tubes, C and D, are drawn to capillaries of very fine calibre, which widen again to the bore of A and B. The tube F, placed on H, and held there by the support N, is removable and can be replaced by any one of a number of similar tubes. By means of the stop-cock Q it is possible to establish or to interrupt the communication between B and the balloon L. The tubes A and B are bent to a right angle at their junction with G. Interposed between the rubber tube K and the balloon L is a glass tube which by an opening communicates with the air.



The method of making determinations is as follows: In the tube B-C-E is a column of distilled water, the left meniscus of which is at O. A tube, F, is filled by capillary action with blood. It is then placed end to end with D, and by means of suction produced by L the blood column is drawn up to O. The cock, Q, is then opened, and by the suction of the bulb the water and blood flow through the tubes A and B. As soon as the blood reaches i, suction is discontinued, and the readings are made. The body of water which has risen in B gives the relation of the viscosity of the blood in question to that of distilled water. The water and blood are now expelled by pressure on the bulb L, and the cock is closed when the water reaches O, F is removed, and the tube DA is cleaned by drawing ammonia through it twice.

If the blood is very viscid or coagulates rapidly, it may be drawn to  $\frac{1}{2}$  or  $\frac{1}{4}$ , and the values obtained multiplied by 2 or 4 respectively.

Controls made with fluids of known viscosity are accurate to within 0.5 per cent.

Experiments by Hess showed that with a rise in temperature of  $1^{\circ}$  C. the viscosity decreased 0.8 per cent. Observations at temperatures of ordinary rooms show an error of about 4 per cent.,

which, when compared to other errors which the personal equation may introduce, is negligible. The only correction of importance is that necessitated by great variations in the temperature.

The blood is obtained by a needle stab in the lobule of the ear, which has been previously cleaned with alcohol. In those cases in which determinations on the plasma are to be made the blood is drawn from the median basilic vein by venepuncture, coagulation retarded by the addition of dry hirudin, and the blood sedimented.

The viscosity of the blood in health is a variable factor. It is slightly greater in men (4.55) than in women (4.51); it depends on the number of red corpuscles, the hæmoglobin contents, the gaseous richness, and, to a lesser degree, on the proteid, fat, and salt in the blood. And yet it varies directly with none of these factors. The viscosity of the normal plasma varies from 1.7 to 2.0, average 1.86.

The results which Austrian\* obtained are as follows: The viscosity of the blood and of the plasma is reduced in the anæmias, both the primary and the secondary. As the blood becomes regenerated the viscosity becomes normal. In leukæmia there is hypoviscosity of the blood with hyperviscosity of the plasma. The viscosity of the blood and of the plasma is increased in polycythæmia. Hypoviscosity of the blood and hyperviscosity of the plasma are almost constant in cases of nephritis, the former being due to anæmia, the latter to retained products of metabolism. Hypoviscosity occurs often, though not always, in cases with hypertension. In cardiac diseases without œdema no constant change in viscosity is to be found, the coefficient apparently varying with the anæmia and with the carbondioxide content of the blood. In cardiac cases with hydræmia there is hypoviscosity of the plasma. In diabetes mellitus the viscosity of the blood and that of the plasma are increased. This may be explained as the result of hyperglycæmia, and of lipæmia, and of the concentration of the blood due to the polyuria. In icterus there is generally an increased viscosity of the blood and of the plasma, probably the result of cholæmia. In typhoid fever the viscosity varies with the anæmia. It is increased by hydrotherapy, and apparently is uninfluenced by diet. The  $\left(\frac{Hb}{V}\right)$  quotient is oftener decreased than increased. In pneumonia the viscosity is generally above normal. This may be due to cyanosis and to the retention of salt. Here, too, the  $\left(\frac{Hb}{V}\right)$  quotient is low. In malarial fever the viscosity of the blood is usually normal or subnormal, rarely above normal. The viscosity of the plasma is normal or increased if hæmoglobinæmia is present. In no disease can a pathognomonic alteration in the viscosity of the blood be demonstrated.

HÆMOGLOBINÆMIA is a condition in which there is free hæmo-

\* Johns Hopkins Hosp. Bull., Jan., 1911, vol. xxii, p. 9.

globin in the plasma of the circulating blood. It is demonstrated by examining the spectrum of the plasma obtained by centrifugalizing the fresh blood, or the spectrum of the serum after the corpuscles have separated by clotting.

When wholesale destruction of red blood-cells takes the form of a fragmentation of the corpuscles, some of the fragments may be picked up by the spleen (causing acute "spodogenic splenic tumor") and other internal organs, while others allow their hæmoglobin to dissolve in the plasma, causing hæmoglobinæmia. This form sometimes follows severe skin burns. Again, many of the corpuscles may "lake" in the blood stream and then will be seen at the same time in the plasma many "complete shadows" and the free hæmoglobin. To explain so extensive hæmolysis there, the blood must contain some cythæmolytic poison, as certain blood poisons, or the poisons of acute infectious fevers, mentioned on page 248, or the malarial parasite. In paroxysmal hæmoglobinuria one assumes that there is an antecedent hæmoglobinæmia, and yet this can seldom be proved, although a lowered resistance of the cells to mechanical injury has been demonstrated. The explanation of paroxysmal hæmoglobinæmia (and the resulting hæmoglobinuria) would seem to be as follows: While in the blood of 25 per cent. of all persons there is an isohæmolysin which is capable of dissolving blood-cells, not the cells of their own blood, or those of other individuals belonging to the same group with themselves (the groups determined according to the isoagglutination reaction), but capable of dissolving the blood-cells of certain individuals of other groups, patients with paroxysmal hæmoglobinuria have also in their plasma a hæmolysin of amboceptor-complement nature which differs from other isohæmolysins in that it is capable of dissolving the corpuscles of these patients' own blood as well as those of other individuals, and which needs for its action a low temperature followed by a high temperature (Moss). When hæmoglobin is free in the plasma it is transformed in the liver to bile pigment as rapidly as possible, causing hypercholia and often a definite jaundice; while if the destruction of red cells involves at least a sixth of their total number hæmoglobinuria is the result.

METHÆMOGLOBINÆMIA is the condition in which the circulating blood contains methæmoglobin. It may result from the action of certain poisons, such as potassium chlorate, antifebrin, acetanilid, etc. In the blood of these patients, the pigment is both free in the plasma and intracellular. In certain "idiopathic" cases it is associated with weakness, cyanosis, and diarrhœa, and in these cases the methæmoglobin is entirely intracellular. The presence of this pigment is proved by finding in the spectrum of the blood, which has been sufficiently diluted with water, not only the two bands of oxyhæmoglobin, but also a band in the red which extends from  $\lambda 620$  to  $\lambda 645$

(shading off on the two sides to  $\lambda 615$  to  $\lambda 650$ ), and which promptly disappears on the addition of ammonium sulphide.

**THE BLOOD IN CARBON MONOXIDE POISONING.**—In severe cases of carbon monoxide poisoning a diagnosis is sometimes suspected from the macroscopic appearance of the blood, especially of the venous blood, which has a slightly brighter red tint than normal. A positive proof of its presence is made by examining with the spectroscope a few drops of the blood sufficiently diluted with water. The bands of carbon monoxide hæmoglobin closely resemble oxyhæmoglobin, except that they are slightly nearer the violet end of the spectrum and do not unite to the single band of reduced hæmoglobin on the addition of a little ammonium sulphide. Sahli warns us against placing too high an estimate on the test. Men are so susceptible to this gas that they may show severe symptoms of poisoning before the detection of carbon monoxide hæmoglobin is possible, while a positive test will soon disappear after the patient has breathed fresh air for a little while.

**SULPH-HÆMOGLOBINÆMIA**, first distinguished from methæmoglobinæmia by van der Bergh, has attracted considerable attention since the paper of West and Clark.\* The sulph-hæmoglobin is contained in the corpuscles and is not free from the plasma. No free  $H_2S$  can be demonstrated in the blood. This condition is often associated with cyanosis, headache, great weakness, and obstinate constipation.

The blood spectrum of cases with sulph-hæmoglobinæmia shows the two bands of oxyhæmoglobin and a band placed somewhat like that of the methæmoglobin, but not quite so far in the red, since it extends from  $\lambda 610$  to  $\lambda 625$  and does not disappear, is, in fact, even intensified on the addition of ammonium sulphide, while the bands of oxyhæmoglobin unite to the one band of reduced hæmoglobin.

**BILIRUBIN AND UROBILIN IN THE BLOOD.**—For the detection of bilirubin and urobilin in the blood, Conner and Roper † suggest the following modification of Syllaba's Method.

Five cubic centimetres of clear blood serum, diluted with 10 cc. of distilled water, after the addition of about 0.5 gm. of powdered sodium sulphate and 1 cc. of five per cent. acetic acid, are coagulated by short heating on a water-bath. The filtrate from this is either almost colorless or faintly pink. The color of the filtrate does not always indicate the presence or absence of urobilin, and one must carefully examine all filtrates spectroscopically after adding two or three drops of Lugol's solution, using a large spectroscope and absorption cells from 1 to 4 cm. in depth. (A small pocket spectroscope gives fairly satisfactory results if filtrates are clear.) After neutralization the filtrate is tested with Schlesinger's zinc-acetate solution

\* *Medico-Chirurgical Transactions*, vol. xc, 1907.

† *Arch. of Int. Med.*, 1908, vol. ii, p. 532.

for confirmatory green fluorescence, and is allowed to stand twenty-four hours before being pronounced negative. The precipitate, usually very faintly yellow or white, is boiled a few minutes on a water-bath with from 20 to 30 cc. of 5 per cent. acid alcohol (hydrochloric acid, 5 parts; 95 per cent. alcohol, 95 parts). The filtrate from this is sometimes colorless, sometimes green, sometimes yellowish pink. The colorless filtrates contain neither urobilin nor bilirubin; the green filtrates contain bilirubin, and on spectroscopic examination occasionally show a band of urobilin; the pink filtrates contain only urobilin.

THE QUANTITATIVE DETERMINATION OF BILIRUBIN IN THE BLOOD.—Conner and Roper recommend a slight modification of Gilbert's method. This method is used in testing different dilutions of the blood serum for Gmelin's test, which is supposed to fail to be positive in dilutions of over 1:40,000 of bilirubin. This method is based on the assumption that the limit of the Gmelin reaction in the blood represents a definite strength of bilirubin, and that albumin, hæmoglobin, indican, and lutein do not interfere with the play of colors. In fluids rich in albumin the complete Gmelin reaction, that is, the characteristic play of colors, occurs only when bilirubin is present in relatively strong concentrations, for example, between 1 to 3,000 and 1 to 5,000. In dilutions of from 1 to 7,000 to 1 to 11,000 the reaction appears as a distinct bluish-green ring, but without the color play. In weaker solutions of bilirubin the blue ring at the point of contact becomes finer and has a violet rather than a green reflection, but remains distinct up to a dilution of about 1 to 40,000.

A series of dilutions of known strength of the blood serum to be tested is made in eight or ten flat-bottomed, cylindrical glass tubes of standard size. They are 4 or 5 cm. long and have an inside diameter of 1 cm. (It is convenient to have a block of wood or a frame in which these tubes can be set in a row.) Three pipettes are necessary. One holding 1.5 cc. and graduated accurately to  $1/20$  cc. is used for measuring the blood serum; one holding 2 cc. and graduated to  $1/4$  cc., for measuring the diluting fluid ("artificial serum"); and one with a tapering point, for measuring approximately  $1/4$  cc. of the nitric acid reagent. Two reagents are required. In making the artificial serum the whites of several eggs are added to an equal volume of 0.7 per cent. sodium-chloride solution. These are thoroughly mixed and allowed to stand on ice for twenty-four hours. The liquid is then decanted, and to it is added caustic soda, in the proportion of 0.3 gm. to 100 cc. It is then allowed to stand three or four days, during which a precipitate forms which includes the coloring matters of the egg-white. When used the liquid should be 1 cm. thick and perfectly colorless. This artificial serum in its fluidity, albumin content, and alkalinity approximates blood serum.



It should be kept cool and should be freshly made at least every month. The nitric-acid reagent counts of 200 cc. of pure  $\text{HNO}_3$ , 100 cc. of distilled water, and 0.06 gm. of sodium nitrate. The blood serum is from 15 to 20 cc. of blood obtained by venepuncture and allowed to clot.

Into each of 6 glass tubes introduce with the second pipette exactly 0.5 cc. of artificial serum, add with the first pipette increasing amounts of blood serum (*e.g.*,  $1/20$  cc. in the first tube,  $2/20$  in the second tube,  $3/20$  in the third,  $5/20$  in the fourth,  $8/20$  in the fifth, and  $12/20$  in the sixth tube) and mix by shaking. Underlay the fluid in each tube with  $1/4$  cc. of the acid reagent. Or, the artificial serum and the blood serum may be mixed and then introduced into a tube over the nitric acid. This gives a sharper end-reaction.

After standing for thirty minutes the tubes are examined by good daylight (not direct sunlight). The examiner's back should be to the light, his eyes should be somewhat above or below the tubes, not on a level with them, and the tubes should be held against a white background. If the blood serum contains sufficient bilirubin, in some of the tubes at the line of contact between the acid and the serum will be seen a clear blue ring, this being more pronounced in the tubes containing the larger amounts of blood serum. The tube in which the first faint but distinct blue ring is seen, therefore, is assumed to contain bilirubin in the dilution of one to forty thousand.

The strength of bilirubin in the initial blood serum can then be readily obtained by the following formula:

$x = \frac{10 + a}{40,000a}$ , in which  $a$  = the number of twentieths of cubic centimetres of serum used.

Example: If the ring is first seen in the tube to which  $5/20$  cc. of blood serum has been added, then

$$x = \frac{10 + 5}{5} \times \frac{1}{40,000} = \frac{3}{40,000} = \frac{1}{13,333}.$$

The blood serum, therefore, contains bilirubin in the ratio of about 1 to 13,300.

It is important that the examination be made just half an hour after the tubes have been prepared, as the reaction tends to become stronger on standing, and tubes which fail to give a reaction within the specified times may do so after an hour or two. The series of tubes should be so prepared that there will be at least one tube in which no blue line can be seen.

Since the blue ring is found to occur regularly in the undiluted serum of normal persons, Gilbert and Hirscher are led to believe that the serum of normal human blood contains a minute quantity of bilirubin, such as is known to be present normally in the blood of certain of the lower animals. They found this physiologic cholemia to cor-

respond to a bilirubin strength which varies between 1 to 28,000 and 1 to 40,000, with an average of 1 to 36,500.

By this test slight grades of and fluctuations in jaundice are detected and measured, and it has been an aid in distinguishing between appendicitis and cholecystitis.

#### BACTERIOLOGY OF THE BLOOD\*

Before undertaking cultural studies on the blood the observer must have a thorough working knowledge of the principles of bacteriological technique. We shall therefore consider below only such special points as may be useful in the study of the blood.

**Technique.**—The success of blood-cultures is in part dependent upon the obtaining of a sufficient quantity of blood for observation, 15 or 20 cc. being the usual amount withdrawn. In general, the median basilic or cephalic vein is chosen for the operation, the needle being passed through the skin into the vessel selected. If for any reason these be not available, a smaller vein on the dorsum of the hand or foot may be used. Incision of the skin to expose the vein, while practised by some, is not generally to be recommended, as it increases the discomfort to the patient. In very fat or œdematous individuals, however, we may be obliged to divide the skin and subcutaneous fat to find any vein large enough for use. For typhoid cultures in bile mediums where only a small amount of blood is needed the finger-tip or the lobe of the ear may be cleansed with soap, then alcohol and ether, then coated with collodion and the puncture made through the collodion letting the blood drop directly into the tube.

If the cleansing of the surface be carefully carried out, the chance of contamination by skin organisms is negligible.

Ordinarily, the site of operation is scrubbed with green soap and hot water, then rubbed over with Harrington's Solution, washed with ether and alcohol, and then covered with a wet bichloride (1 in 1000) compress. If there be haste and the usual materials for cleansing wanting, the skin may be painted thoroughly with the tincture of iodine until a deep brown, then rubbed clean with 95 per cent. alcohol and the culture made as before. This is quite as satisfactory as the more elaborate method.

The syringe should be of the usual antitoxin type, and have a capacity of 20 cc., and a glass barrel which is perfectly true.

The packing of the piston should be of asbestos and very tight. The Roux or Record syringes are better than asbestos packed ones, but more fragile. Such a syringe may be boiled with impunity. In place of the ordinary washer for the needle a piece of soft black rubber tubing may be cut and, after perforating with a pin, slipped

\* For this section I am indebted to Dr. Thomas R. Boggs.

over the nipple. This withstands boiling longer and gives a tighter joint. A fresh washer should be used for each culture.

The needle should be short and stiff, sharp, and of moderately large calibre, and may be of steel or irido-platinum. To sterilize, the syringe and needle are boiled fifteen minutes, or they may be sterilized in the autoclave. It is well to have a forceps boiled and use this in putting the needle on the syringe. Do not use the syringe until cool.

A moderately tight bandage is placed proximal to the site of operation to distend the vein, and the needle plunged through the skin, which may be anæsthetized with ethyl chloride spray, directly into the vein. The piston is drawn slowly and the syringe allowed to fill with blood. If the bandage is removed before withdrawing the needle, there will be no flow of blood to distress the patient. After withdrawal, the needle and washer are removed and the media inoculated quickly. Always pass the tip of the syringe through the flame of an alcohol lamp before inoculating each tube.

If it is desired to send the blood to a laboratory for inoculation into the proper media it may be discharged into tubes or flasks containing equal volumes of sterile isotonic ammonium oxalate (ammonium oxalate 2, sodium chloride 6, water 1000) solution.

Agar tubes melted and cooled to about 45° C. are used for making plates. Bouillon and litmus milk in flasks containing 100 cc. are preferred for fluid media. Or a number of tubes may be substituted for each flask. The plates should be poured at once. A medium of ox-bile, or of ox-bile and peptone, is now considered best for *B. typhosus*.

The amount of blood in each tube or flask is varied somewhat according to the type of organisms suspected to be present, from equal parts of blood and agar to one volume of blood in five of agar; in flasks, 1 to 2 cc. in 100 cc. of medium. In general, we increase the amount of blood where the feebler growing organisms as gonococcus or pneumococcus are suspected.

The colon group grows better in bouillon, the pneumococcus better in milk. Anaërobic cultures may be made in the ordinary ways.

If after twenty-four hours' incubation the plates show only a few surface colonies, contamination may be reckoned upon. Only deep colonies occurring alike in several or all plates should be used for subculture. True mixed infection in the blood is uncommon. Plates and flasks should be examined daily for five to eight days before discarding as sterile, as small colonies deep in the opaque media may not appear in the first twenty-four or forty-eight hours.

**Value of Blood-cultures for Diagnosis.**—With the increase of laboratory facilities blood-cultures have become much more important in diagnosis. In many instances they afford the only means of accurate diagnosis.

The pyogenic organisms (streptococci and staphylococci) are usually readily demonstrated in cases of general infections, osteomyelitis or malignant endocarditis due to their presence. Some idea of the intensity of the infection may be gathered from the number of colonies per cubic centimetre of blood.

Typhoid bacilli have been demonstrated in the blood in upward of 75 per cent. of a series of cases by Cole, Buxton, Schotmüller, Hewlett, and others, often days or even weeks before the Widal test is positive.

In the paracolon infections the isolation of the organism from the blood or stools forms the only definite means of differentiation.

In pneumococcus infections the percentage of positive cultures is less but still large, the organism being found principally in the graver cases.

Among other organisms of less frequent occurrence in the blood during life may be questioned: *B. aërogenes capsulatus*, *B. coli*, *B. pyocyaneus*, *B. anthracis*, etc.

As blood-culture involves but little inconvenience to the patient, it may be repeated if the first be negative or demand confirmation.

#### AGGLUTINATION PHENOMENA

Through the action of certain bacteria on the tissues there are produced in the blood soluble bodies known as agglutinins. These agglutinins, when sufficiently concentrated, have the property of clumping and rendering non-motile the specific organism whose activities gave rise to their production.

The nature of the interactions between the bacteria and the agglutinating serum is unknown. Theoretical discussion of the phenomena would carry us too far afield.<sup>12</sup>

**Gruber-Widal Test.**—This is the agglutination phenomenon applied to the diagnosis of typhoid fever.

**CULTURES.**—A standard stock culture of *B. typhosus*, which must be actively motile, should be kept for this purpose. An organism cultivated through many generations on artificial media is preferred.

From this stock fresh cultures on agar are grown from twelve to twenty-four hours for use in the test. Some authorities prefer fresh (ten to eighteen hours) bouillon cultures from the stock. Others use bouillon cultures killed by the addition of carbolic acid, formalin, or other toxic substances. Hastings has devised a method, based on analysis of Ficker's "Typhus diagnosticum," which yields very satisfactory, and stable-killed cultures,—viz.: To a mixture of aqueous 5 per cent. carbolic acid, 5 cc., of glycerin, 10 cc., of sterile 0.8 per cent. sodium chloride sol., 85 cc., are added the organism scraped from

<sup>12</sup> For full presentation with literature, see Paltauf, Kolle u. Wassermann's *Handbuch der path. Microorganismen*, Bd. iv. Teil i. S. 645.

two twenty-four-hour agar slant cultures of the typhoid bacillus. The bacilli being gradually and thoroughly rubbed into the solution with a small spatula, allow to stand five or six days before using. This is used by mixing with equal volumes of the diluted sera. Living fluid cultures may give rise to confusion from the presence of clumps due to the growth of the organism. Of the dead cultures, those killed with weak carbolic are preferred, as formalin may cause precipitation of proteids from the serum in flocculi.

The emulsifying of the fresh culture from agar (rather dry slants are best) in 0.8 per cent. salt solution, or in bouillon, seems to offer the most satisfactory results. A loopful of the growth is rubbed against the side of the tube of salt solution until thoroughly broken up and then gradually mixed with the fluid. A loop of standard size is preferred and 1 loopful to 1 cc. of salt solution will give a fairly constant suspension for comparative work. With a little care a suspension free from clumps is easily secured.

**COLLECTING THE BLOOD.**—Glass tubes two inches in length by one-quarter inch in diameter are drawn out into a capillary at either end and kept on hand for the purpose. (See Fig. 101.)

From a free flowing puncture in the ear or fingertip the blood is drawn into the tube by capillary attraction until it is two-thirds full. The tube is then placed flat on a table until the blood has clotted and the serum is separated from the coagulum. The tube is then filed and broken at a point just beyond the clot and the serum withdrawn with a capillary pipette. If a centrifuge is available, the process of separation may be hastened and the yield of serum increased by sealing the tip of tube which is free from blood in a flame and centrifugalizing a few minutes, when the clot and corpuscles will be condensed in the lower end and the serum left as a clear layer above. If it is desired to preserve the specimen or to send it away, both its ends may be sealed in the flame or with sealing-wax. Serum is best kept after separation from the corpuscles in a sterile tube. If larger amounts of blood are required, a vein should be aspirated with the syringe as in the procedure for blood-culture.

**DILUTING SERUM.**—To obtain the dilution of serum used in the reaction a number of methods are employed.

A simple and very satisfactory method is as follows: A piece of one-quarter-inch glass tubing is drawn into a long capillary, as shown in cut. This is plunged into the serum in the collecting tube and the

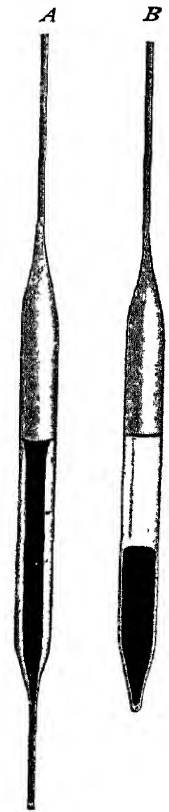


FIG. 101.—Tubes filled with clotting and clotted blood. *A*, blood is clotting spontaneously, the clot now retracting from the sides. *B*, clot in centrifugalized tube.

capillary allowed to fill, care being taken to avoid stirring up the corpuscular layer. From this capillary the serum is dropped into the tubes or dishes in which the dilutions are to be made. A small water-color palette of porcelain is very convenient for making a number of dilutions. Salt-cellars or watch-crystals may be used. As a routine at least two dilutions of each serum should be made, 1 to 50 and 1 to 100.

For this purpose we proceed as follows: To the first drop of serum we add 24 drops of 0.8 per cent. salt solution dropped from *the same pipette*, which has been washed out with distilled water to remove any trace of serum, and then dried in the flame. To the second drop of serum 49 drops of salt solution are added, giving dilutions of 1 in 25 and 1 in 50. Now, the addition to any portion of these dilutions of an equal volume of the suspension of the typhoid culture will give us dilutions of 1/50 and 1/100. In the same way any desired dilution may be made. If greater accuracy or very high dilutions be required, special mixing pipettes similar to the Zeiss mélangeur for blood counting may be employed. Again, dilutions may be made directly from the whole blood with such a mélangeur, using salt solution as diluting fluid and counting each two volumes

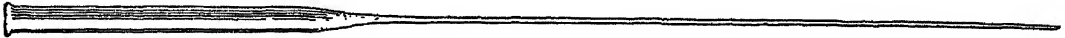


FIG. 102.—Tube used for diluting serum.

of blood as one volume of serum. The mixture is allowed to settle or, better, is centrifugalized before using. With the diluted serum we can now proceed to the macroscopic or microscopic tests.

A. MACROSCOPIC METHOD.—This method depends on the agglutination of the organisms into clumps visible to the naked eye and the eventual precipitation of the clumps, leaving a clear supernatant fluid. The serum is diluted in a test-tube of small calibre, and the organisms added either as a suspension of living or killed cultures; or, what is perhaps more convenient, the full dilution, as 1 in 50 or 1 in 100, is made with salt solution and the organisms from solid culture suspended directly in the diluted serum, as described in the foregoing section. The tube is then examined by strong transmitted light to see that its contents are homogeneous and free from accidental clumps. A narrow band of light from a lamp enclosed by a screen aids in detecting the early appearance of clumping. A positive test is reckoned if there be general clumping at a dilution of 1/50 or higher in one hour with complete precipitation, leaving a clear supernatant fluid after twenty-four hours. The reaction is hastened if the tubes are placed in the thermostat.

This method has the advantage of simplicity in that no microscope is required and that killed cultures may be employed, thus obviating

the necessity for a thermostat and culture media. The "Typhus diagnosticum" of Ficker,<sup>13</sup> now so widely used in Germany, is a preparation of killed cultures, the formula for which is kept secret. More complete details of this method and its results will be found in a recent paper by Borden.<sup>14</sup>

Several pharmaceutical laboratories in this country now make and sell killed cultures for the macroscopic Widal.

B. THE MICROSCOPIC METHOD.—The diluted serum may be mixed with the requisite volume of the typhoid suspension by the use of pipettes, as above noted, and a drop of the mixture taken for observation on a hanging drop slide. Or we may mix the two on the cover-slip directly. To do this we use a platinum loop of stiff wire,

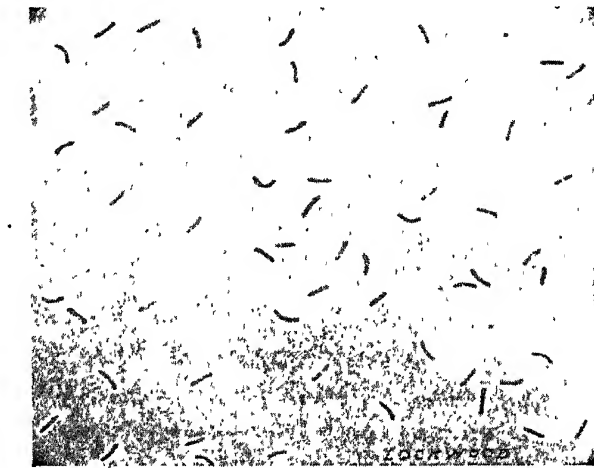


FIG. 103 —Widal test. Field of motile organisms.  $\times 900$ .

the plane of the loop being at right angles to the handle and the diameter of the loop being constant. The loop is dipped vertically into the serum dilution and the drop so obtained placed on the centre of the cover-slip. The loop is flamed off and dipped into the typhoid suspension in the same way, and the two drops thoroughly mixed on the cover-slip. Approximately equal volumes are readily obtained by this simple method, enabling us to secure any desired dilution. The cover-slip is then inverted over the well of a hanging drop slide which has previously been ringed about with olive oil or vaseline, and the preparation is then ready for examination. The hanging drop is observed with a moderately high dry lens (Zeiss D, or Leitz  $1/6$  in.), and is seen best by artificial illumination. The Argand burner or oil-lamp with a yellow flame is preferred. The light is stopped down with the diaphragm so as to bring out the refractivity of the bacteria.

<sup>13</sup> Berl. klin. Wochenschr., 1903, No. 45.

<sup>14</sup> Medical News, March 18, 1905.

The freshly made hanging drop should be free from clumps and show the organisms actively swimming about in addition to their Brownian motion. (See Fig. 103.) After the lapse of one hour, if the test is positive, the organisms will be seen to be collected entirely in clumps and to have lost their motility; this at a dilution of 1/50 or higher. (See Fig. 104.) The presence of a few non-motile free organisms in a field otherwise well clumped is not considered to vitiate the test.

It is frequently noticed that the clumping is better at the higher dilutions, while there may be very marked bacteriolysis at 1 in 10 or 1 in 20 or even higher dilutions. Many normal sera will give perfect agglutinations at 1 in 10, and show no trace of the reaction

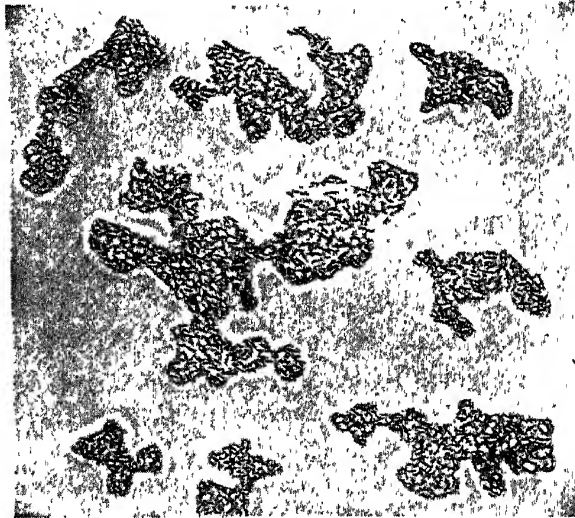


FIG. 104.—Widal test. Field of agglutinated organisms.  $\times 900$ .

at 1 in 50 or higher dilutions. Hence tests based on the low dilutions alone are unreliable. The macroscopic method has rapidly gained favor in the best laboratories, and is probably less open to error than the microscopic, provided strict limits of time and dilution (one hour at dilution of 1 in 50 or higher) are observed. There is so much variation in the determination of microscopic clumping by different observers that it is often difficult to compare their results. Some authors consider any aggregation of a very few organisms agglutination. These differences have led to much confusion, particularly in experimental work.

**AGGLUTINATION WITH DRIED BLOOD.**—This method is based on the use of blood dried on glass, tin-foil, or glazed paper, and is only accurate where the blood is carefully weighed and the dilution based on weight instead of volume. Its sole recommendation is the convenience with which the blood so dried may be transported.



**Value of Agglutination Reactions in Typhoid Fever.**—While the Widal reaction rarely fails to appear in typhoid fever, it may be long delayed and is not often present before the seventh or eighth day, so that it may be no aid to early diagnosis. Still, it remains our most certain confirmatory test after the bacilli have disappeared from the circulating blood, and is indispensable in abortive, doubtful, and obscure cases.

The persistence of the agglutinative reaction is variable, the limits being from a few weeks to many years. Some cases of long persistent Widal have been attributed to the presence of typhoid bacilli in the gall-bladder, in gall-stones, or in the urinary bladder.

The agglutination of *B. typhosus* by normal sera at the standard dilution, 1/50 in one hour, is so rare as to be negligible.

The question of "associated agglutinations" in which the serum agglutinates two or more organisms closely related, as *B. coli*, *B. alkaligenes*, and *B. typhosus*, is too complicated to find place here. Suffice it to say that the limited time and the high dilution employed in our tests are sufficient to give us reliable specific results.

**Paracolon Infections.**—While these often give highly specific agglutinations, the presence of associated agglutinins should be considered and the diagnosis of any one type of paracolon by agglutination reaction only would be questionable unless cultures were made for confirmation.

**Other Agglutinations.**—The agglutination reactions have been applied to many different organisms with more or less definite results, but in most cases they have not reached any considerable diagnostic value and are often very difficult of application.

Those specially interested will find full details in the references appended.

Dysentery group: Flexner, *Bull. Johns Hopkins Hosp.*, 1900; also *Centralbl. f. Bakt.*, 1901, Bd. 30. Shiga, *Centralbl. f. Bakt.*, 1878, Bd. 23, 24.

Tubercle bacillus: Arloing and Courmont, *Compt.-rend. Ac. de sc.*, 1898, t. 127, p. 312; *Zeitschr. f. Tuberkulose*, 1900, Bd. i. H. 1, 2. Fränkel, *Hy. Rundschau*, 1900, No. 13.

*Streptococcus*: Van de Velde, *Arch. de Méd. exp.*, Paris, 1897. Neufeld, *Zeitschr. f. Hygiene*, 1903, Bd. 44.

*Meningococcus*: Jager, *Zeitschr. f. Hyg. u. Inf.*, 1903, Bd. 45.

Malta fever: Wright, *Lancet*, March 6, 1897. Strong and Musgrave, *Phila. Med. Journ.*, 1899. In Malta fever the reaction is on a well-established practical diagnostic basis.

Paracolon: Korte, *Zeitschr. f. Hyg.*, 1903, Bd. 44. Coleman and Buxton, *Am. Jour. Med. Sci.*, 1902. Schottmüller, *Deutsch. med. Wochenschr.*, 1900, p. 511.

## RED BLOOD-CELLS.

The erythrocytes are specialized non-nucleated cells, which consist of hæmoglobin, 95 per cent., and stroma, and whose chief function is to carry the oxygen to the tissues, and to a lesser degree the carbon dioxide to the lungs.

In **shape** they are circular, discoid cells, which in well-made fresh specimens lie flat. In many of them a biconcavity is apparent, but in normal blood this must be looked for pretty sharply, and in many cells is not seen at all; in some conditions, especially the secondary anæmias, it is very evident. The opinion of Weidenreich and Lewis, that in the circulation these cells are not flat but are cap-shaped, is borne out in many specimens, especially those from the bone-marrow; clinically it is a point of no importance. Cells of normal blood, unless subjected to considerable mechanical injury in making the smear, are perfectly round and of a size varying from 6 to 9 microns in diameter. When they are not round, or are of very abnormal size, the term "poikilocyte" is applied to them (Plate I, 25-28). Such cells occur in pernicious anæmia especially, even of a mild grade, and in other anæmias of severe grade, especially in cases of cancer, tuberculosis, etc. They are probably due to alterations in the plasma.

**Structure.**—These cells are about the hardest of all to study, being so sensitive. Various methods have been applied to demonstrate their structure, and each has shown a different one. Foà's description, a peripheral structureless hæmoglobin-containing layer, a middle with a net-work of fibres enclosing granules, and a centre of homogeneous protoplasm, is one of the most elaborate and often quoted. The consensus of opinion now is that all of the fibres, layers, etc., are artefacts; that the various granular-like bodies seen in the fresh cells are not an essential part of the cell; that those in the stained are, some at least, precipitates of the fixing agent, or of the stain; and that structure, although it certainly exists, is yet to be demonstrated. Ehrlich considers that heat is the best fixative, because it gives a homogeneous cell without structure. This argument seems weak, for heat renders difficult of observation the structure of many other cells of similar nature, including the protoplasm, but not the granules, of leucocytes, and hence may be the very worst method for the study of red cells.

Not only is their fine, but also their coarse structure in dispute. The cell membrane formerly believed to exist, then doubted, is again claimed by Deetjen, who describes it as elastic, gelatinous, glassy, and stained best in underheated specimens; while others claim merely a hæmoglobin-free concentration of the stroma at the surface.<sup>15</sup> Since so many believe the nucleus to disappear within the cell, they think it necessary to find some remains of it there. The question of the nucleoid is in dispute, some considering it to be related to the nucleus and others to be totally independent.<sup>16</sup> The word "nucleoid" has a variety of meanings in the writings of at least six observers who have employed it. It means among others the "differentiated inner body of Löwit;" that is, a nucleus-like structure in the centre of the red blood-cell which in certain specimens is very apparent, it taking a basic stain; it has a fibrillar structure, and a central clear space; in its centre again is a differentiated inner body, which "may be extruded as a platelet." "The nucleoid develops after the extrusion of the nucleus (Maximow)," but Löwit considered it the remains of the now invisible nucleus. Against these as parts of the cell is to be urged that constant technique is necessary to give constant results; that their size varies from very small to that two-thirds of the corpuscle; their periphery is indistinct often, and radially striated; that is, they do not look "genuine" It is hard to believe that Maximow can tell the age of red cells by this inner structure.

The non-nucleated red blood-cells when fresh certainly look structureless. Al-

<sup>15</sup> See Peskind, *Am. Jour. Med. Sci.*, 1904, vol. cxxiv.

<sup>16</sup> Maximow, *Arch. f. Anat. u. Physiol.*, 1899.



## PLATE I.

### CELLS OF NORMAL BLOOD.

1. Small lymphocyte; small mononuclear.
2. Eosinophile leucocyte.
- 3, 4. Large lymphocytes.
5. Transitional mononuclear.
- 6, 7. Polymorphonuclear neutrophile leucocytes.
8. Mastzell.

### CELLS FOUND IN SPLENOMYELOGENOUS LEUKÆMIA.

- 9, 11, 17. Neutrophile myelocytes.
10. Dwarf polymorphonuclear neutrophile leucocyte.
- 12, 13. Transitional cells between myelocytes and polymorphonuclear cells.
14. Eosinophile leucocyte.
15. Lymphocyte.
- 16, 19, 20, 21. Large mononuclears.
18. Dwarf polymorphonuclear eosinophile leucocyte.
22. Polymorphonuclear eosinophile leucocyte.

### ERYTHROCYTES IN CHLOROSIS.

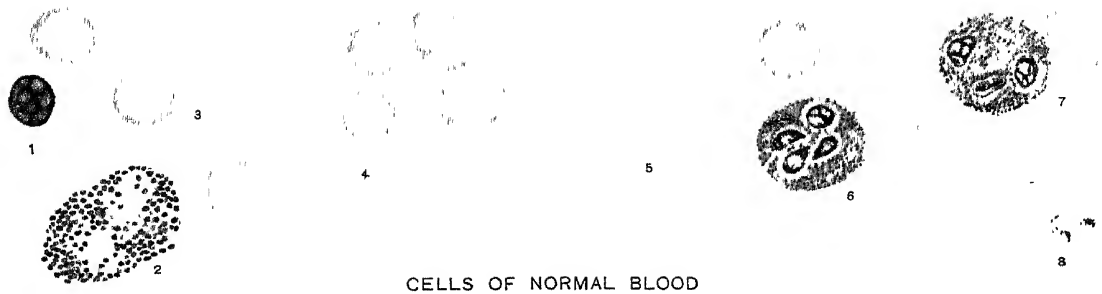
23. Cells found at the height of the disease. These are the "doughnut" or "pessary" forms.
24. Cells from the same case as 23 during convalescence.

### POIKILOCYTES IN PERNICIOUS ANÆMIA.

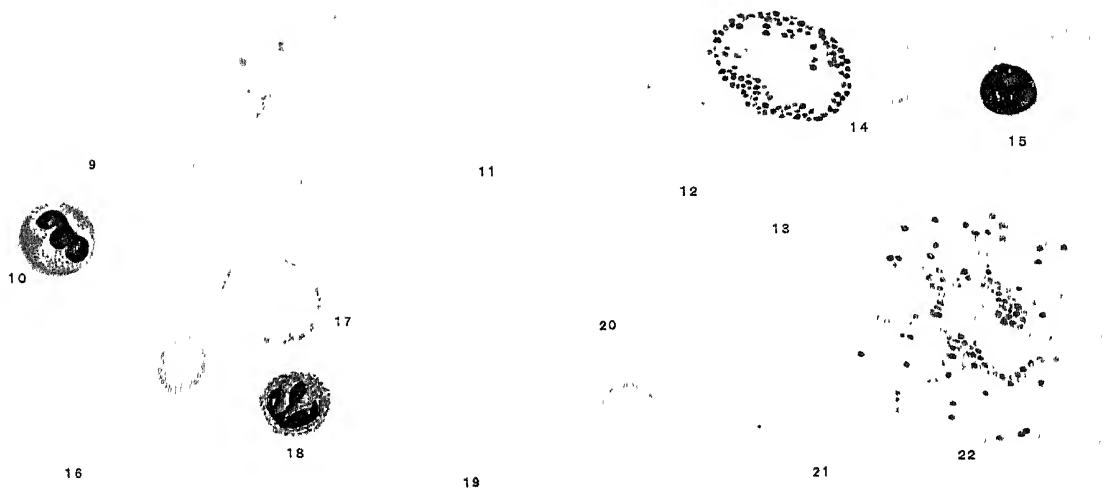
25. Battle-door form.
26. Sausage form.
27. Microcyte.
28. Megalocyte.

### NUCLEATED RED BLOOD CELLS.

29. Mature normoblast.
30. Immature normoblast.
- 31, 32. Intermediate forms.
33. Megaloblast.
34. Normoblast with nucleus showing fragmentation or incomplete mitosis.
35. Fuchsinophilic normoblast.
36. Leucocyte of the same size as 37 shown for comparison
37. Large nucleated red cell.
38. Intermediate nucleated red (or megaloblast), the "reptilian form."



CELLS OF NORMAL BLOOD



SPLENO-MYELOGENOUS LEUKÆMIA.

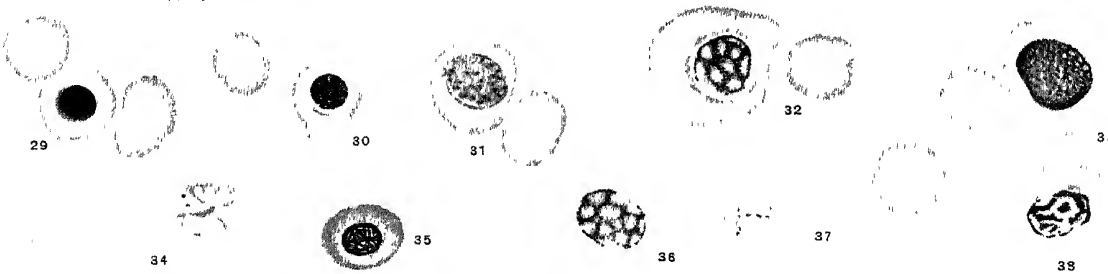


AT HEIGHT OF DISEASE.

ERYTHROCYTES  
IN CHLOROSIS.

SAME CASE DURING  
CONVALESCENCE

POIKILOCYTES  
IN PERNICIOUS ANÆMIA.



ALL STAINED WITH EHRLICH'S TRIPLE STAIN  
AND DRAWN TO SAME SCALE.

LEUCOCYTE FOR  
COMPARISON.  
NUCLEATED RED BLOOD CELLS.



most every staining method will give a different result, and yet it is certain that they have some structure, that they have a discoplasm or the "oikoid;" a stroma which may be seen after the hæmoglobin has been washed out; but the various "inner bodies," etc., seen may be nothing but masses of degenerated protoplasm, the Maragliano endoglobular degenerations, and in the light of present knowledge all may be considered artefacts. Only perfect cells are seen in the circulation. They enter it as almost perfect, and they leave it before marked signs of age are apparent (see page 509).

**Size.**—In the adult these cells vary from 6 to 9 microns in diameter with an average of 7.5 microns. Hayem found that 75 per cent. varied from 6.6 to 8, 12.5 per cent. from 6 to 6.6, and 12.5 per cent. from 8 to 9 microns in diameter. These limits are quite fixed in the adult, although a very few dwarf cells do occur at all ages. But in the normal infant blood the cells vary much more, with normal limits of 3.3 to 10.3 microns. In disease the adult blood may resume this infantile condition. Evidence is given that the red blood-cells of various nationalities differ somewhat, the size diminishing as one approaches the equator. In the fresh blood occur physiological rhythmical changes, the cells being somewhat larger in the venous than in the arterial blood where they are charged with oxygen (Hamburger). Pathologically, they vary much in size.

**MICROCYTES.**—This term means cells under 6 microns in diameter. The smallest are about 3.5, and yet some are 2.2 microns. There is doubt whether all these are perfect cells or are schistocytes,—*i.e.*, fragments of larger cells,—since the process of constriction of small fragments from the red blood-cells can be followed in the fresh blood and be produced by pressure on the cover-glass, etc. For the cells above 3.5 microns in diameter we can find nucleated reds of corresponding size which represent young forms. These are seen in perfect fresh specimens. Such cells have no biconcavity as a rule, are spherical, and hence have a deep color. They occur normally in the embryo infant, and especially in premature children. In these cases they are often polychromatophilic. They are found rarely in the healthy adult, but are common in all anæmias, especially the primary and severe secondary anæmias.

**MACROCYTE** is a term applied to cells from 9 to 12 microns and above in diameter; for cells from 12 to 16 microns, the term **MEGALOCYTE**; and for those above 16 microns, **GIGANTOCYTE**. These cells occur in largest numbers in pernicious anæmia. Some believe that if 10 per cent. of the reds are macrocytes a diagnosis of pernicious anæmia is justified. They also occur in leukæmia and in chlorosis. In chlorosis they are often very pale, hence the term "chlorotic" or "dropsical" cells. They are also very common in cases with cholæmia, which is of interest since cases of pernicious anæmia are so often jaundiced (Osler). That their size is due to hydræmia is considerably

in dispute; it is well known that plasma is quite constant in its water-content, and that the variations in water of the total blood affect especially the cells. Whether the chief difference between the small dark cells and the large pale cells is the amount of water, each having approximately the same amount of hæmoglobin, is the question. In pernicious anæmia the largest cells are sometimes the darkest, and some of the microcytes are exceedingly pale, while in secondary anæmia the reverse is true. (See Plate I.)

**Staining Properties.**—Red blood-cells like all other cells while alive are achromatophilic, and take a stain only in proportion to their death changes. If the red cell is killed by a good fixative which prevents post-mortem changes, all normal cells are monochromatophilic, and since they take only acid stains from a mixture, are *acidophilic*. With most dyes it is the hæmoglobin especially that takes the stain, and changes in this will show themselves to a certain degree by the tint and the tone which the cells take.

Cells which take other than the acid component of a stain either as a whole or in part are termed *polychromatophilic* or *basophilic*. By this term (a synonym of which is “anæmic degeneration”) we do not now include the basophilic granules to be described later. Eosin stains the basophilic cells more faintly than the normal, and if followed by a basic stain, such as hæmatoxylin, will be supplanted by it. A basic stain should not color a normal red unless it is in an old dried smear. Ehrlich’s triple stain is unsuitable, since methyl green is too feeble a basic stain, and the acid components too much in excess to show the basophilia well.<sup>17</sup> Basophilic corpuscles are usually larger than the normal, have less biconcavity, and often are poikilocytes. With hæmatoxylin and eosin such cells take a violet tint; with the Ehrlich, a fainter tone than normal, or a grayish color; with polychrome methylene blue stains a bluish violet; in all cases, basophilic.

Ehrlich explains basophilia as a coagulative necrosis. In favor of this are, that other signs of degeneration are also present; that it can be produced in animals by inanition; that these cells are present within twenty-four hours after a hæmorrhage, that is, before any nucleated cells or other signs of active regeneration have appeared; and that it affects especially the megaloblasts. The other view is that they are young cells. Others say that the granules are evidence of incomplete intracellular oxidation. Basophilic cells occur in pernicious anæmia; in the grave secondary anæmias, especially those due to cancer; in the eruptive fevers, malaria, the purpuras; and after various blood poisons.

Other cells are “*fuchsinophilic*” (Plate I, 35); that is, stained

<sup>17</sup> See Walker, J. of Bost. Soc. Med. Sci., November, 1899.



with Ehrlich's triple stain they are very red. Since so many of the nucleated reds of the marrow are fuchsinophilic this is considered a sign of a young cell. (These cells are usually distorted, as if very soft.) The same is said of the basophilic cells, for nearly all nucleated red cells are slightly basophilic, but basophilia and fuchsinophilia are not the same.

While the middle ground is usually unsatisfactory, yet there is, we think, the best of evidence that young red cells in general are basophilic, and good evidence that a degenerating cell will usually take such stains. The same may be said for microcytes, macrocytes, and the basophilic granules; it is hard to say whether they are signs of regeneration or degeneration, but since they occur in such a variety of conditions it is improbable that they have always the same significance. They are not signs simply of anæmia, for anæmia may be severe without them, yet in general all abnormal corpuscles—abnormal in size, shape, amount of pigment, nucleation, with protoplasm having abnormal staining affinities, etc.—are grouped as “anæmic forms,” and the explanation is of little importance. To us basophilia as evidence of youth is interesting, since it was Theobald Smith who first suggested it in a case of purpura and later emphasized it in his studies on Texas fever.<sup>18</sup> Walker found such cells in the normal blood of all lower vertebrates; and in the foetus of the dog and guinea-pig even ninety times as many as in the blood of the mother. In normal marrow the basophilia is in inverse proportion to the amount of hæmoglobin that the cells contain, judging by the fresh specimen before staining, hence the term “anæmic degeneration;” but this lack of hæmoglobin could be primary or result from the loss of some from the cell.<sup>19</sup> Walker suggests that these “cells hurried into the circulation while too young” are as good an index of anæmia as the blood-count, and their detection much easier. Germani also emphasizes them as an important feature of the blood picture in severe anæmias, their number being in direct proportion to the severity of the case, and, since easy to stain, suggests them as a valuable hint in diagnosis and prognosis.

Engel's opinion that the fuchsinophilic cells represent a related yet different type from orthochromatic cells (*i.e.*, cells staining in the usual manner), and that they send cells into the blood-stream only in anæmia when the supply from the orthochromatic nucleated reds is exhausted, has not received much support. Taylor says fuchsinophilic cells are not found in embryonic or infantile blood. We would object to this most emphatically, since that is where we have found the best illustrations of these cells.

<sup>18</sup> Walker, *loc. cit.*

<sup>19</sup> See also Stengel, *Contrib. from Pepper Lab., Univ. of Pa., 1900.*

*Partial polychromatophilia* is best illustrated by the Maragliano endoglobular degeneration (see p. 454), these degenerated areas staining well with a strong basic dye, especially methylene blue. The probability is that many of the so-called "inner bodies," "nucleoids," and other so-called evidences of cell-structure are nothing but these stained areas of degenerating protoplasm, which sometimes resemble malarial parasites, and the extrusion of which gives rise to bodies resembling platelets. Their resemblance to malarial parasites is so striking that we know one eminent pathologist who admitted that had he not been sure that before death a patient had not malaria he would have been unable to say that the inclusions in certain red blood-cells stained with hæmatoxylin and eosin were not malarial parasites. These degenerations certainly led to many mistaken diagnoses before the chromatin-staining mixtures were used.

The ring bodies described in some of the red cells of anæmic blood by Cabot,<sup>20</sup> and which require for their demonstration the polychrome methylene blue-eosin mixtures, are, he suggests, nuclear remains. They are red rings, ovals, or bands, evidently not related to the stippling of the cells. They occur especially in pernicious anæmia, but also in the leukæmias and various secondary anæmias.

In heated specimens, if this be done too quickly, or if they be exposed to moisture, around the periphery of the cell may sometimes be seen a row of large dots, which are neither true granules nor do they resemble the granules in malaria.

In certain cases of malaria (those we have seen have all been tertian and from the Tropics) the infected cells show a remarkable granulation (Plate III, 10, 13). They contain granules which are of quite uniform size, which are as coarse as the eosinophile granules, and stain purple in the Hastings' stain, while the rest of the cell stains paler, in fact may be almost colorless, as if the hæmoglobin had been condensed into these dots. (This is merely a "descriptive explanation.") We have seen cells in which the granules appeared hung in a hyaline envelope around the parasite. They can be seen in the fresh unstained cell; the lead granules cannot (Boggs). We are sure these are not artefacts, and that they are not the same as the granules of lead poisoning which also may be present in the cells. One who has seen both will not identify them.

The "methylene blue degeneration of Ehrlich" is the name given to a beautiful picture seen in specimens of fresh blood stained by this dye, the cell containing a mesh-work of fibres.

"VITAL BLOOD-STAINING."—To study these granules and fibres in the unfixed cells, one puts on the wet smear a granule of methylene blue or neutral red, then the cover is sealed at once to the slide with

paraffin, and the beautiful threads of fine granules are soon seen. Whether these are preformed, or are signs of death or degeneration, or merely precipitates of stain in the cell, is uncertain.

Another excellent method of vital blood-staining was used by Rosin.<sup>21</sup> A cover-glass is lightly spread with the saturated alcoholic solution of methylazur or of toluidin blue, which is then allowed to dry. Over this stained surface a blood smear is made, and the surface at once inverted over a hollow slide with vaselined rim. The blood can be watched for as long as twenty-four hours. These methods are not used nearly enough.

Various poisons, potassium chlorate, pyrogallie acid, *et al.*, often will produce vacuole-like areas or clumps in the cells, which are motile and which may break free from the cell, or the cell may be dissolved, leaving them free. Heinz and Bloch describe these as "areas of poisoned protoplasm."

THE BASOPHILIC GRANULATION OF GRAWITZ (Plate II, 22, 24, 25).—In certain conditions, especially lead poisoning, pernicious anæmia, leukæmia, etc., certain of the red blood-cells when stained with any good basic stain, particularly gentian violet or methylene blue, contain minute granules. They are not seen in fresh unstained specimens, and do not increase after the blood has stood for some time.

While any methylene blue-eosin mixture will do, the most beautiful specimens are prepared as follows. The air-dried smears are fixed for from three to five minutes in absolute alcohol, washed in water, and while still wet are stained with Löffler's methylene blue for a few seconds or much longer, then dried, or examined in water. The bluish-black granules stand out against the clear green corpuscles.

A beautiful stain to differentiate these from fragments of the nucleus, which many suppose them to be, is that of Pappenheim (Boellke)

STAIN I. Acid. carbol. liquefact., 0.25; aqua dest., 100; methylene green (pur.), 1.

STAIN II. Acid. carbol. liquefact., 0.25; aqua dest., 100; pyronin (pur.), 1.

Fifteen cc. of I. and 35 cc. of II. are well mixed and filtered. The blood-smear fixed by heat (not alcohol) is stained for a few seconds with the filtrate. The fragments of nuclei are deep greenish-blue, the granules bright red.

In a severe case one finds as many as five or six of these "stippled cells" in a field, but, as a rule, several fields must be searched in order to find one. There may be but one or a few granules in a cell, but as a rule it is well sprinkled, and to such a degree that some consider that the tone of a polychromatophilic cell is due to them. They may be from dust-like size to a micron or more in diameter. They occur anywhere in the cell, but are distributed quite regularly as a rule. Many think they are situated in the external layers of the protoplasm. They occur in the severest anæmias, especially the primary pernicious, in which they are large and conspicuous; in sec-

<sup>21</sup> Rosin and Bibergeil, *Zeitschr. f. klin. Med.*, 1904, vol. liv. p. 107.

ondary anæmia due to cancer, especially of the gastro-intestinal tract; in cachexia; in leukæmia, in which cases they are not numerous; and in septic processes; while in chlorosis some say they are rare, others (Stengel and Pepper, in 11 of 18 cases) common. They also occur in phthisis, lues, chronic parenchymatous nephritis, small contracted kidney, cirrhosis of the liver (Grawitz). In gout they are many in number, and yet in rheumatism with even severe blood changes they are very rare. Few are found in tuberculosis, typhoid fever, pneumonia, lues, nephritis, etc. In gout it is of interest that especially large numbers are found in those cases with hæmatoporphyrinuria; Guyot found them regularly in the hæmoglobinuria due to cold; in tuberculosis Grawitz says they occur only after the secondary infection of a cavity. That condition in which they occur in the largest numbers is lead poisoning. They are found in the blood of Europeans who have recently moved to the Tropics.

Grawitz interpreted them as areas of coagulated necrosis and they commonly now bear the name "Grawitz basophilic granular degeneration."<sup>22</sup> White and Pepper,<sup>23</sup> Stengel and Pepper,<sup>24</sup> Bloch, and others agree.

On the other hand they are normal in embryonic blood, never, some say, in adult blood; nucleated reds often contain them, good evidence against their relation to a degenerating nucleus. They are often present in the degenerated reds, but also occur independently of other degenerations, as polychromatophilia, poikilocytosis, etc. Their relation to malaria and to polychromatophilic degeneration is now generally abandoned. Others say that they are in some way or other related to new formation of cells, while now and again recurs the view that they are related to the nucleus.

Cadwalader distinguishes three groups of granulated corpuscles; those with the granules in fine and coarse thread-like strands; those with fine dot-like granulations; and those with dense coarse masses. The first type is found in small numbers in normal blood; the second, the most common form, in lead poisoning and pernicious anæmia; the last in those cases of lead poisoning in which nucleated reds are plentiful. These last granules, in position and size, suggest a breaking-down normoblast nucleus. The reds are otherwise normal. Others deny that transitional stages between fragmenting nuclei and these occur, and point out that they are least in the bone-marrow where karyorrhexis is most common. Again, it is claimed that they do not occur until definite signs of regeneration have also occurred (but the hydræmia is also at its maximum then), well seen in post-hemorrhagic anæmia, hence the opinion of some that they are related to regeneration. In favor of this is the difficulty of producing them by the direct influence of lead salts.

Cadwalader<sup>25</sup> finds them always associated with nucleated reds in lead poisoning, and thinks them the result of a fragmentation of the nucleus of the red cell. In favor of this is the fact that the increase in nucleated reds precedes that of the granulated cells, and he thinks the forms of granules suggest steps in the process.

At this point we wish to state that those dealing with these granules do not exclude any other basophile granules, hence practically every granule found in red blood-cells is described under this one title. In our opinion there are at

<sup>22</sup> Hamel, *Deutsch. Arch. f. klin. Med.*, May 23, 1900.

<sup>23</sup> *Am. Jour. Med. Sci.*, September, 1901.

<sup>24</sup> *Am. Jour. Med. Sci.*, May, 1902.

<sup>25</sup> *Bull. of the Ayer Clin. Lab., Univ. of Pa.*, January, 1905.

least three different basophilic granulations in red blood-cells, that these when compared side by side have little resemblance the one to the others, and, we suspect, no relationship; but the scope of this book could include a discussion of the latter point only in so far as it emphasizes their appearance or occurrence.

The malarial granules are described on page 510. Compare them side by side with the Grawitz granules, and they do not seem to belong to the same class of structures. Grawitz believed them different, but did not state reasons. The one cannot be seen in the fresh cell, the other can.

The granules described by Vaughan (see page 456) as remnants of nuclei do not resemble the Grawitz granules, and yet from Cadwalader's figures we judge he includes them as his coarse variety. It is impossible to say they are not related, but they do not look as if they were.

Perhaps their greatest importance is in lead poisoning, since here they may be the only abnormal blood-feature. In other diseases in which they occur, as the anæmias, they form but a minor part of the blood picture, although they occur in large numbers. They are very fine in size. Some claim that they can be found in the blood of all lead-workers. This may be the case, but it depends on the length of time that the specimen is studied, and we do find cases of lead poisoning, particularly the peripheral neuritis cases, the one condition in which it would be most important to find them, in which they have not been found, or only one cell, in the time at the disposal of the ordinary clinical worker. They are especially numerous in cases with gastro-intestinal features, to which symptoms they bear a rough parallelism, but this may be better explained by the fact that both are early features of lead poisoning. They vary much in number from day to day. As a rule they appear very early, even after four days' exposure to lead, and they may be present in the blood of those exposed over twenty years. They are the first sign of the anæmic blood changes, and the last sign to disappear, hence we may speak of an anæmia even before the count drops. In the diagnosis of intestinal colic they may be of importance, but in the cases of peripheral neuritis we have failed to find them so.

Other references to this subject are, Naegeli,<sup>26</sup> who considers them related to blood regeneration; Boellke,<sup>27</sup> who denies that they bear any relation to the nucleus.

**Number of Red Blood-Cells.**—The average count for the normal adult man is usually given as 5,000,000 cells per cubic millimetre of blood; for the woman, 4,500,000. In a healthy young man, however, it is more common to find from 5,000,000 to 6,000,000.

By *polycythæmia* is meant a condition with more cells per cubic millimetre than this present; by *oligocythæmia*, one with a smaller number. It is evident that this number is simply relative, that variations may be due either to actual variations in the number of red blood-cells in the body, or to the amount of plasma, which may by diluting the blood cause an oligocythæmia, and when it is reduced in amount, a polycythæmia.

The blood-count may vary in different parts of the body. Oliver<sup>28</sup> found that anything which increases the blood-pressure even locally will cause a rise in the count at that point; as, for instance, in a limb that has been hanging in a dependent position, active or passive motion,

<sup>26</sup> Munch. med. Wochenschr., 1904, No. 5.

<sup>27</sup> Virch. Arch., 1904, vol. clxxvi. S. 47.

<sup>28</sup> Brit. Med. Jour., 1896.

digestion, etc. These variations are, however, quite slight; yet exercise will raise the count, the local application of cold and of heat lower it or raise it, according to the production of stasis, vasodilatation, or constriction.

Excessive exercise, Willebrand found, would raise the count of red cells from 3 to 23 per cent. (average 12.3 per cent.), and of leucocytes from 19 to 97 per cent. (average 47 per cent.).

PHYSIOLOGICAL VARIATIONS.—The effect of *sex* has already been mentioned. This variation occurs only during the menstrual period of life, since for girls until their fifteenth year the count averages 5,444,000, while for boys of the same age 5,102,000; between the ages of forty and sixty, again the count of women averages 5,000,000.

The count varies much with *age*. The maximum is at birth, in which case it may be even 7,000,000, but, as a rule, is lower,—e.g., 5,740,000 (Stengel and White). Otto found the average for the first four days to be 6,155,000; in one child ten hours old, 6,910,000. It depends somewhat on the time at which the umbilical cord is tied, since by tying late there may be a gain of almost one million cells per cubic millimetre. After the first four days the count begins to drop, and is at a minimum in about one year. These high counts at birth are probably due to the concentration of the blood; the body is not yet accustomed to its environment, and loses considerable water. They last but a few days, not over ten, after which nucleated reds also disappear.

From birth until about the tenth year the count reaches the minimum, then slowly rises. There is considerable difference of opinion when this minimum occurs. Gundobin gives the average count during this period as 5,100,000. It rises from puberty to thirty years of age, during which period young healthy persons often have from 5,500,000 to 6,000,000 cells. From about thirty to fifty years, 5,000,000 for men, 4,500,000 for women, may be considered normal, and after forty the count is inclined to slowly drop in men and rise in women.

Not satisfied with the age curves usually quoted in text-books, we have attempted one, using the material of this clinic, especially the neurasthenics and a few patients with apparently normal blood. In addition to this we have the very valuable studies of our medical students on their own blood, counts made to conform to the most rigid criteria of accuracy (see page 469), as accurate, we believe, as any which have yet been published.

We have used means, not averages; this is, we think, the correct way of arriving at a fair estimate of blood-counts, etc., for the extremes should not be considered when the question is of the most common condition. All the figures are arranged in order of magnitude, and that chosen as mean around which the greatest number clusters. For a discussion of the low hæmoglobin estimations, see page 529.

*Blood of Patients.*

## MALES.

Years.	Cases.	Reds (mean).	Hb mean Per cent.	Index.	Leucocytes.
6 to 15	5	5,560,000	85	....	7500
16 to 25	36	5,200,000	85	0.8	6500
26 to 35	69	5,300,000	90	0.85	7000
36 to 45	42	5,500,000	90	0.82	5500
46 to 55	21	5,300,000	80	0.75	9000
56 to 65	9	5,000,000	80	0.8	....
66 and over	5	4,000,000	60	0.77	7500

## FEMALES.

10 to 15	5	5,000,000	75	0.75	8000
16 to 25	43	4,500,000	77	0.85	7500
26 to 35	55	4,500,000	80	0.88	7200
36 to 45	34	4,600,000	72	0.80	7700
46 to 55	17	4,500,000	77	0.85	7000
56 to 65	10	4,500,000	70	0.78	6000
66 and over	3	4,700,000	65	0.7	7000

The students' counts showed the following: age, twenty to twenty-five years; males, 176 cases; mean of reds, 5,000,000 (extremes 4,500,000 and 6,700,000); 14 (8 per cent.) were below 5,000,000, and 15 (8.5 per cent.) above 6,000,000; of leucocytes, 7500 (52 cases); of hæmoglobin, 14.5 gms. (Miescher), 92 per cent. (Fleischl), 95 per cent. (Dare), 92 per cent. (Gowers).

Females, 16 cases; mean of reds, 4,800,000; of leucocytes, 8000; of hæmoglobin, 11 gms. (Miescher), 85 per cent. (Fleischl), 87 per cent. (Dare), 82 per cent. (Gowers).

*Nutritional Conditions.*—In thin muscular persons the count is somewhat higher than in the stout. A large meal may cause a temporary slight decrease, said to be due to the increased fluid of the plasma. During hunger periods there is an increase, a rise of a half-million cells in twenty-four hours being common, attributed to concentration of the blood.

The *temperature* has an influence on the count. In winter there are about 500,000 more cells per cubic millimetre than in summer (this was well seen in some of our students' counts). The change of residence from temperate zones to the Tropics may lead to a drop in the count of from 500,000 to 2,000,000 cells.

*Pregnancy.*—For both mother and the foetus there is said to be a diminution in the count during the last part of pregnancy; for the mother a drop of about half a million cells and 20 per cent. of hæmoglobin; for the foetus of from seven and a half to eight and a half months the count was found to be 7,000,000, while at nine months 6,500,000 (Biondi and Gardini). The blood of mother and child are, on the whole, rather independent; in case the mother has an anæmia-producing disease the child can preserve its count fairly well, and *vice versa*.

Thompson made a very careful study of twelve cases in Dr. Williams's clinic of this hospital. He found a moderate decrease in the reds from the fourth to the eighth month. The count and hæmoglobin rise to normal at term. The specific gravity shows the most striking curve parallel to that of reds and hæmoglobin, but more accentuated, with the initial fall and terminal rise, the minimum (1040.8) at the sixth month.

*Altitude.*—A great amount of work has been done to decide this, one of the most vigorously debated chapters of hæmatology. The rise in count in persons ascending to high altitudes is a phenomenon long ago witnessed, but its explanation is not yet wholly clear. The count increases as persons ascend at the rate of about 50,000 cells per one thousand feet, and diminishes as soon as they descend, or at the latest thirty-six hours after. The increase in the count is especially marked after a sudden ascent to a considerable height; there is little rise from an ascent of 1200 metres, it is slight and tardy after an ascent of 1800 metres, but immediate and considerable after an ascent of 3000 metres. The rise is certainly more rapid than could be explained by a new formation of blood, and on the descent there are no signs of blood destruction. The rise is best seen in invalids, especially those with lung tuberculosis. The symptoms of anæmia are even aggravated.

There would now seem to be two factors which enter into the case,—the first a temporary one, due to a changed distribution of the blood-cells, and later, in eight or ten days, a permanent change due to a true new formation of cells.

Miescher and his pupils consider that the diminished oxygen tension is the stimulus to new blood formation, confirmed by the work done in many laboratories and by many men, Jaquet *et al.* Evidence of an increase of blood was found in animals kept for several days in an atmosphere of reduced tension, or by keeping these animals at high altitudes. And yet practically all of this experimental work has been challenged, and animals sent to high altitudes give disappointing results.

Other explanations were given:—that there was a concentration of the blood due to evaporation (Grawitz), hardly possible, since the solids of the plasma do not change as much as the count; that it was an accumulation of cells in the capillaries (Tuntz); that the cells lived longer; that there was an initial fragmentation of the reds causing the early increase, and then a true new formation (Koppe); that there was a peripheral vasoconstriction causing a concentration of the blood from the increased lymph formation (Bunge); and lastly, that it was due to the error in the instrument, the reduced atmospheric pressure affecting in some inexplicable manner the thickness of the stratum of blood counted (Gottstein). So many papers have recently appeared on this subject that we shall mention but a few, especially that of Campbell and Hoagland,<sup>20</sup> who consider that the change is due simply to a changed distribution in the blood-cells, depending on the lowered blood-pressure, this due to the lowered barometric pressure, and to a compensatory increased heart action, hence the pulse increases almost parallel to the count. Mosso showed peripheral vasodilatation, hence stasis in dilated capillaries, at altitudes. The heart will compensate all these factors if the person remains at an altitude, hence later the count returns to normal. The difference in temperature has some influence; hence the count at Colorado Springs is about 800,000 lower than at the city of Mexico, two places of the same elevation. Some consider this the chief factor (Weinzirl, *et al.*). Experiments with rabbits showed a decreased count in the mesenteric circulation corresponding to the rise in the peripheral circulation.

<sup>20</sup> Am. Jour. Med. Sci., November, 1901.



The experiment of Gaule, who studied his blood during a balloon ascension and found the rise accompanied by the appearance of many nucleated reds, has not been confirmed.

**DRUGS AND THERAPEUTIC MEASURES.**—Among the drugs which increase the count by their effect on the reds are iron, which is almost a specific in chlorosis, affecting especially the formation of hæmoglobin, and arsenic, a drug equally valuable in pernicious anæmia, which seems to affect the production of the red blood-cells. Mercury in large doses causes an anæmia. The destruction of weakened cells by this drug may explain the Justus test for lues. Lead causes a chlorotic anæmia, explained by some by the gastritis, by others by its direct injury of the red blood-cells. In favor of the latter view is the granular degeneration so constant in these cases.

Any drugs which affect the amount of plasma by causing rapid losses of fluid to the body, as diuretics, emetics, purgatives, diaphoretics, will cause a rise in the count, providing the change be sufficiently rapid. Yet one is usually disappointed in the slight effect of these drugs.

Cold baths cause an average increase of 1,860,000 (Thayer). This is due to the peripheral vasomotor constriction, hence stasis in the capillaries. The specific gravity also is increased. The maximum increase is immediate, and disappears in about one hour. Breitenstein thinks the effect of a cold bath is greater for a typhoid patient than for a normal man, since the distribution of cells is already abnormal.

There is often a transitory post-operative rise of 100,000 to 1,000,000 cells, probably a peripheral phenomenon.

**PATHOLOGICAL CONDITIONS.**—The acute cachexias of infectious disease, due to the toxins of certain of the specific fevers, can cause a marked anæmia, even when there are no hemorrhages. This is true in certain cases of pneumonia and of typhoid fever, but is by no means common. With the lowering of the count there is increased pigment in the urine and increased globulicidal properties of the serum. Hence there is probably an increased destruction of the red cells.

**Chronic Cachexia.**—Of this tuberculosis, cancer, and lues are the best illustrations. (See pages 624, 638, and 643.) The methæmoglobin-producing poisons diminish the count, because of their direct destruction of the cells. Among these are pyrogallie acid, the chlorates, certain of the coal-tar products, as antifebrin and phenacetin.

**POLYCYTHÆMIA.**—In this section we follow closely the excellent article by Watson-Wemyss.\* Polycythæmia, an increase in the number of red corpuscles per cm. of blood, may be transient or permanent, and permanent polycythæmia may be secondary or primary. A *transient polycythæmia* is usually due to a concentration of the blood caused by loss of fluid from the plasma. It exists in

\* Edinb. M. J., Feb., 1911, vol. iv, N S., p. 129.

cyanosed parts especially, also in cases of acute phosphorous (red blood-corpuscles even 8,650,000) and of carbon monoxide (red blood-corpuscles even 6,630,000) poisoning. Vomiting cannot explain all of these high counts.

*Permanent or absolute polycythæmia* would seem to be the result of a true increase in production of new red cells. In favor of this view are the signs of active blood formation (nucleated cells, polychromatophilia, leucocytosis, eosinophilia, etc.). This polycythæmia may be a secondary process (erythrocytosis) or a primary process (erythræmia).

*Erythrocytosis* is a permanent polycythæmia the cause of which is in part at least understood. Among these causes are: high altitudes; diseases of the heart, especially congenital heart disease, in which the count may vary from 8,000,000 to 9,000,000 per cmm., mitral-valve disease, and adherent pericardium; lung diseases, especially empyema, acute miliary tuberculosis, and pneumonia; chronic stimulation of the bone marrow by poisons, as phosphorus, acetanilide, etc.

*Erythræmia* is considered a primary disorder, since we cannot yet call it secondary to any known cause. It seems due to abnormal activity of the marrow, and is often accompanied by enlargement of the spleen, cyanosis, and sometimes by arterial hypertension. This condition has been named Vaquez's disease, Osler's<sup>30</sup> disease, and splenomegalic polycythæmia, myelopathic polycythæmia, and cryptogenetic polycythæmia. The red-blood count varies from 6,000,000 to 13,000,000. These cells are of normal size and nucleated red cells are not rare. Poikilocytes and polychromatophilic cells are sometimes seen. A polymorphonuclear neutrophile leucocytosis (from 20,000 to 91,000) is the rule, although a leucopenia has been found. There is also an absolute eosinophilia. Myelocytes are rarely found.

Osler reviewed nine cases, four of which he reports. The cyanosis was extreme, lasting even for years. The highest count was Koester's of 13,600,000, and but one was below 9,000,000 (8,250,000); hæmoglobin, 120 to 150; specific gravity, 1067 to 1080; leucocytes, 4000 to 20,000, but most below 10,000. Zamfirescu reports the case of a woman with polycythæmia, cyanosis, dyspnœa, and cough. Kikuchi reports a case with bronchiectasis. Türk<sup>31</sup> reports seven cases like Osler's, two with autopsy, with counts from 7,700,000 to 10,600,000. He suggests that it is due to a primary hyperplasia and increased function of the erythroblastic myelogenous tissue, hence is analogous to leukaemia. Other interesting cases of polycythæmia without cyanosis occur, as Zandy's,<sup>32</sup> who proposes the term "erythrocytosis." Türk<sup>33</sup> reports a case of polycythæmia with cirrhosis of the liver and enlarged spleen. Gresböck<sup>34</sup> mentions cases with nothing but the high count.

There is one point to be borne in mind in this connection, that we usually count capillary blood, not arterial or venous, and the count

<sup>30</sup> Am. Jour. Med. Sci., 1903, vol. cxxxvi.

<sup>31</sup> Wien. klin. Wochenschr., 1904, Nos. 6 and 7.

<sup>32</sup> Münch. med. Wochenschr., 1904, No. 27.

<sup>33</sup> Deutsch. med. Wochenschr., 1904, No. 50.

<sup>34</sup> Deutsch. med. Wochenschr., 1904, No. 20.

in the capillaries need not be the same as that in the vessels. A capillary field of the frog's mesentery or rabbit's ear, *e.g.*, is seen to contain few corpuscles moving in single file through the capillaries, and some channels so narrow that the cells do not enter them at all. In cases of active congestion from warmth, or of venous stasis, the capillary bed is much widened, the capillaries filled with cells, even those which before transmitted only plasma; hence the count is higher. It is not so much changes in the relation between plasma and tissue lymph which can cause very rapid changes in the capillary count while that in the arteries may remain constant, as factors governing filling and circulation of the capillary area. The count in capillaries and veins is about the same. Following more marked changes in temperature, as *e.g.*, after a cold bath, the count does rise in the arteries, since then the flow to the tissues is increased.

These changes are much more marked in the leucocytes than in the reds, since the former collect in the vessels, forming layers along the walls.

Cyanosis may deceive one much, as, for instance, in a case with normal blood-count which at autopsy shows a condition suggesting pernicious anæmia. The same is true of certain dysenteries.

Similar cases with high counts follow the use of various coal-tar products.

A student recently handling aniline oil became cyanotic; red cells, 5,900,000; hæmoglobin, 107 per cent (Dare); leucocytes, 6100. Six days later the reds were 5,084,000, hæmoglobin, 78 per cent. (Dare).

**Resistance of the Red Blood-Cells.**—Many methods have been proposed for determining the resistance of the red blood-cells, in the hope of explaining phenomena such as hæmoglobinæmia. At first these methods were mechanical, chemical, electrical, but now are biological.

**Hamburger's Method.**—The resistance of the red cells is usually tested by exposing the washed red cells to hypertonic and hypotonic salt solutions and noting the point at which hæmolysis takes place. Sixteen small test-tubes are used, each containing 1 cc. of a sodium chloride solution, the lowest a 0.4 per cent. solution, and each succeeding one 0.03 per cent. higher than the preceding one. One drop of a suspension of the washed corpuscles (in the original method of Hamburger one drop of the patient's blood is used) is added to each, and the tubes, after a gentle shaking, are allowed to stand six hours. The normal blood plasma is isotonic with a 0.9 per cent. NaCl solution, but the normal corpuscles are not laked unless the concentration is less than about 0.4 per cent. In congenital family cholæmia the hæmolysis may begin at 0.7 or even at 0.9 per cent. The method is faulty, however, since in paroxysmal hæmoglobinuria the cells have been found normal, but of lowered resistance to mechanical disturbance. Stengel proposes the following method. The blood is diluted 1:10 in a Ziess leucocyte pipette, with sodium chloride solutions varying from 0.42 to 0.52 per cent. The blood is mixed and then blown into small tubes sealed at one end. These are allowed to stand and then centrifugalized for from two to five minutes, then held against a

white paper to see in which the corpuscles have laked. The advantage of this method is that more fluid is used, and that the percentage is not materially altered by the salts which escape from the cells. Stengel considers it is not osmosis alone, but a chemical or a vital reaction between the stroma and the Hb, which holds them together. As a result of his experiments he found that saturation with an excess of carbon dioxide (as in congestion) causes no great morphological change and no particular alteration in the cells, and yet the vulnerability is greater; that cold produced marked changes, as, for instance, if the congested finger be frozen, which is suggestive concerning hæmoglobinuria; that hypotonic salt solutions have the power in the test-tube as well as within the blood-vessels of decolorizing and vacuolating red blood-corpuscles. This point is disputed by many workers, who find that even the intravenous injection of distilled water does not lacerate any blood-cells. Heat of even slight degree changes the shape and size of the corpuscles and finally decolorizes them. A higher degree causes budding, vacuolation, and a somewhat higher degree complete fragmentation (see page 451).

**Mechanical Influences.**—In some conditions the cells have been found to have varying resistance to shaking. Meltzer has shown<sup>35</sup> that the effect of shaking depends upon the rapidity of vibration, that for each blood there is a minimum and maximum rate which the cells can bear without destruction. Laker has tested the cells by passing the discharges of a Leyden jar through them. Various other methods have been proposed, but with as yet little result.

**The Estimation of Hæmoglobin.**—This should be the easiest and the most useful determination in blood-work, and it is unfortunate that the use of faulty instruments has resulted in the accumulation of a vast amount of data of very little value; for the estimation of hæmoglobin is of more importance than the blood-count, as it is so much less time-consuming. In the case of the blood-count we know, supposing the work has been neatly done, what is meant by the figure given; but for hæmoglobin we must know the kind of instrument used, its make, and the amount it has deteriorated. In general we know remarkably little that is accurate about the hæmoglobin content of the blood in disease.

It is unfortunate that instruments do not all read in grammes rather than percentage, for their makers do not agree what quantity of hæmoglobin should be called normal, nor is there a figure which is normal for all ages, since the age curve of hæmoglobin is very hilly. If instruments are to read percentages, it would be well to have one for each of the various periods of life, since the blood of a normal child of about ten years would read but 80 per cent. on an instrument standardized for a normal man of thirty years. Were hæmoglobin expressed in grammes, there would be less danger of calling such a child "slightly anæmic;" it would also be easier to restandardize an instrument.

Another element of error is that instruments are standardized against hæmoglobin in dilute water solution, while hæmoglobin in an albuminous fluid like the blood-plasma will give higher readings; hence in reading the blood of extreme anæmias we may get misleading figures. For instance, in post-hemorrhagic anæmia the hæmoglobin would seem to drop much lower than is really the case.

In judging the various instruments, one should consider the principle on which the instrument is constructed, and whether or not the maker has followed this principle well. For instance, the v. Fleischl seems well made, but there are several inaccuracies in its principle; the Gowers instrument has fewer inaccuracies, but is so poorly made by many manufacturers that it has fallen into disrepute.

<sup>35</sup> Johns Hopkins Hosp. Rep., vol. ix.

**Miescher's Modification of the Fleischl Instrument** (see Fig. 105).—This is at present our best instrument. It is, however, for laboratory use only, since it is expensive, bulky, and requires a dark room, considerable time for each determination, and considerable practice. The blood is mixed in a beautifully made pipette (see Fig. 106), which allows dilutions of 1:200, 1:300, or 1:400. The markings of these pipettes are particularly good, especially the small lines on either side of each main line, each indicating  $\frac{1}{100}$  of the length of the column between the tip and the "1" mark, thus obviating the necessity of losing valuable time in trying to bring the blood column exactly to a mark. The polished conical end is also an advantage.

A large drop of blood is aspirated to the point indicated for the desired dilution. Such a dilution should be chosen as will allow the

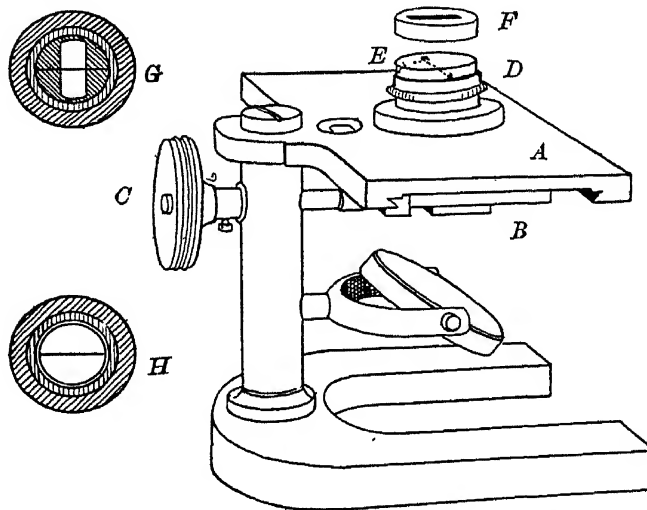


FIG. 105.—Miescher's modification of Fleischl's hæmoglobinometer. *A*, stage; *B*, color-prism rack; *C*, milled head; *D*, cell; *E*, cover-glass; *F*, cap; *G*, cell seen from above; *H*, cell of Fleischl's instrument.

readings to be made near the middle of the color-prism. A normal blood cannot be diluted to the 1:200 mark, since the readings will be "off the scale" of the table of equivalents. The diluting fluid used is 0.1 per cent. sodium carbonate. (A stock solution of 10 per cent. is diluted one hundred times.) Distilled water has been recommended, but with the dilute soda solution we get a color-tone more approximating that of the prism, which materially aids the readings. The blood is mixed exactly as for a blood-count, is well shaken, then the contents of the capillary tube are blown out. In filling the cells (of which there are two, one 15 mm. and the other 12 mm. in depth) it is essential first to fill one side with water, to make sure that there is no leakage into the other half. The blood, well shaken, is blown into the other chamber of the cell. Both the water side and the blood side should have convex meniscuses. The cover-glass, *E*, is then slid on

carefully, pushing off this excess and leaving the chambers exactly full. The slightly raised partition prevents mixing. The small cap, *F*, is then put in place, to hold the cover-glass secure and also to limit the field of vision to one about  $3^\circ$  in length. The cell is then placed in the receptacle on the stand, *A*, and the instrument placed in a screen which admits the light at one point only, where it will fall directly on the mirror and illumine both sides equally. The light to be used is a yellow flame, whether from gas, oil, or candle. Electric

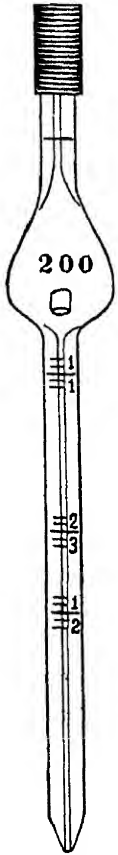


FIG. 106.—Mixing pipette of Miescher's hæmoglobinometer.

light, a gas-light with a mantle, or sunlight, cannot be used. The person should sit in a comfortable manner with the eyes about 25 cm. above the instrument, and make his readings with both eyes open. The milled head, *C*, moving the color-prism, is then rotated until that part of the prism (see Fig. 107) which just matches the color of the blood-mixture is under the water half of the cell. In doing this it is well to make quick excursions to both sides of the point, gradually diminishing them until the point of matching is reached. Since the retina is soon fatigued, and is not then sensitive to colors, the eyes should be rested after each fifteen seconds of color-matching. A very conscientious, painstaking student will sometimes get results much worse than the careless student, since through careful work the eyes are fatigued. When the color is matched the reading is made. At least five such readings should be taken,—ten are better,—and the mean, not the average, used. The blood is then removed by sucking it up with the mélangeur from this (the deep) chamber, the shallow chamber filled in the same way, and a similar series of readings is made. Since these cells have heights which are to each other as 5 is to 4, and since different parts of the color-prism are used, if the readings of the lower multiplied by  $\frac{5}{4}$  differ from the average made with the higher by not over 2 per cent., and the instrument is a well standardized one, it is seen that the readings are so

controlled that considerable error at this point is impossible. We insist that the student shall if necessary put the blood back again into the deep chamber and repeat the work until the readings with the two cells, both calculated for the 15 mm. cell, do not vary over two points.

The great advantage of the instrument is that each is accompanied by a scale which gives the number of milligrammes of hæmoglobin per litre of diluted blood corresponding to the readings of that particular instrument. It is of the utmost importance that the right book be used. It is then easy, making due allowance for the dilution, to

determine the number of grammes of hæmoglobin in 100 cc. of blood, the desired result. If then, with due observance to the age curve, the worker wishes to express his answer as a percentage, he is at liberty so to do. This instrument has been found to be correct within 0.2 per cent. of hæmoglobin. In case a light screen is not at hand, a tube of blotting-paper may be fitted over the cell, thus the side light

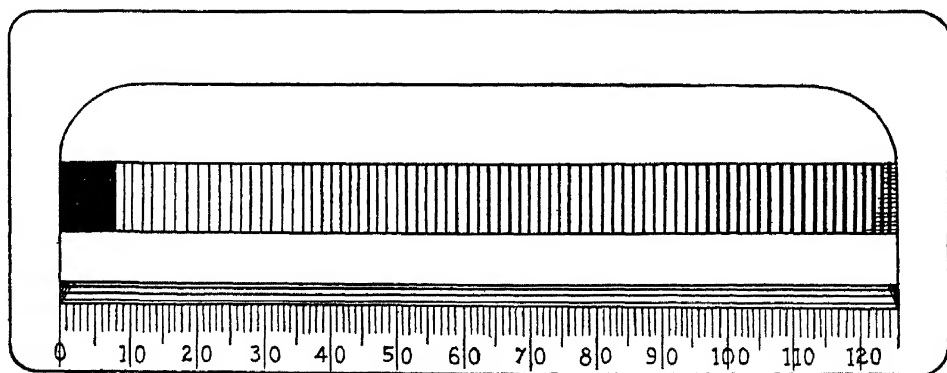


FIG. 107.—Color-prism of the Fleischl-Miescher instruments.

is screened from the eye over this tube. A person should be careful in this case to use the eyes alternately, so that neither may become fatigued.

The mixing pipette is cleaned, etc., just as is that used in blood-counting.

**Fleischl Hæmoglobinometer.**—This, until within a few years, was in this country the favorite instrument. The Miescher machine just described is an improved form of this. The blood is taken in a small short cylindrical capillary tube (see Fig. 108), which is only a few millimetres long, perhaps 1 mm. wide, and holds from about 5 to 8 cmm. of blood. It is fastened in a small metal holder by means of cement. To wash this in alcohol or ether results invariably in loosening the tube, and when once loosened it is so small and easily broken that the pipette is as good as ruined. It should be cleaned in water, and then by drawing through it a needle with a thread soaked in alcohol and then in ether. It must be perfectly dry. The point of prime importance is that the number on the handle of this pipette shall correspond to the number on the post of the machine, otherwise gross errors will certainly result. This pipette is filled by touching its end to a large drop of blood. It should not be stuck into the blood, since any wetting of the outside is to be avoided. One makes sure that there is no blood on the outside, and that the tube is so exactly filled, that the surface of the fluid is flat. Meanwhile,

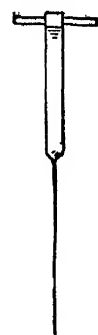


FIG. 108.—Pipette of the Fleischl instrument.

the cell (see Fig. 105) of the instrument has been filled on the one side with water and on the other side by a few drops of water. The pipette is dropped into this latter and emptied by rapidly agitating it in this water; then with a few drops of fresh water the drop of fluid clinging to it is washed back into the cell. By means of the handle of the pipette the blood is then thoroughly mixed with more water, until this chamber of the cell is filled to the brim. The two halves should then be exactly full, without concave or convex meniscus, and certainly without any leakage from one side to the other. They may be covered over with a suitable cover-glass. In a dark room the instrument is read as the Miescher.

There are a few precautions to observe. The images of the two chambers should fall on the right and left halves of the retina, never on the upper and lower, since the lower half of the retina is not nearly so sensitive as is the upper; the light should never be in front of the instrument, but at the side; as small a candle as possible should be used; if there is no screen handy, a tube of dark paper will suffice to cut out extraneous rays. The inconveniences of the machine are the following: in the first place, one is looking at a color field of the prism, which varies at its extremities by at least  $15^{\circ}$ , and the observer must try to read the color at the centre of a field with such wide variation as this (compare the cells *H* of the Fleischl with *G* of the Miescher). It is difficult to see how a person can claim to make readings within 2 per cent. Again, the instrument has certainly not been standardized as accurately as is desirable, there being considerable difference between the older and the newer instruments, and even in the latter it is stated that the prism has straight sides, which certainly does not fulfil the requirements of a good optical instrument, since depth of color is not directly proportional to thickness of glass. For this reason all readings should be made on the upper half of the prism; hence, if the blood be known to be anæmic at least two or three pipettefuls are employed for each determination. The instrument is bulky; it is also expensive. The greatest objection to it that we find is that it has the appearance of accuracy without the reality. A person that has used no other machine is usually confident that he can read within at least 2 per cent. We suspect that the error inherent in the machine is at least 5 per cent. The observer should be very careful to use his retina not over fifteen seconds at a time, to prevent fatigue. To clear the blood in case of lipæmia and high leucocytosis by means of ether and potassium hydroxide is not to be recommended.

**Gowers' Instrument.**—This little instrument (see Fig. 109) is much to be recommended, perhaps is the best for the general practitioner. It is cheap, easily portable, simple, and should be fairly accurate. It consists of a color-tube, *B*, containing a fluid the tint of one per cent. hæmoglobin solution, and a graduated test-tube, *A*, into which 20 cmm. measured in the pipette, *C*, are diluted with water until the tints match. The percentage is read directly on the graduated scale from the height of the diluted blood.

The blood is drawn to the proper mark in a measuring pipette, and then blown into the graduated tube, in which have previously been placed a few drops of distilled water. By sucking this water



back and forth the inside of the pipette may be quite thoroughly cleansed of the blood, but it should then be again filled with distilled water and this added to wash out the last trace. The blood is mixed with the water by covering the end of the tube with the thumb and inverting it several times, but the thumb should be wiped across the top of the tube, that the clinging drop of water may not be lost to the mixture. It is well in reading these two tubes to use both direct and transmitted light. They are best held against a sheet of white

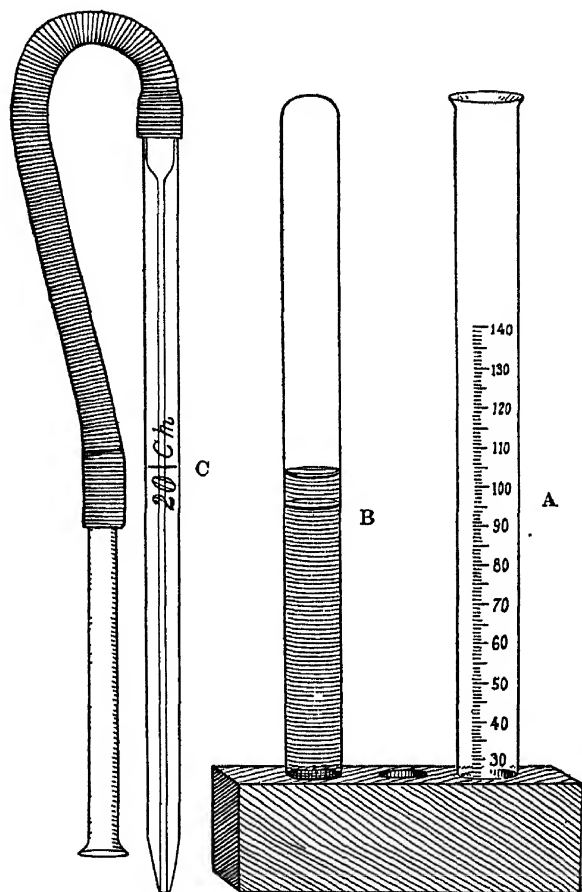


FIG. 109.—Gowers's hæmoglobinometer. A, graduated tube; B, color-tube; C, pipette.

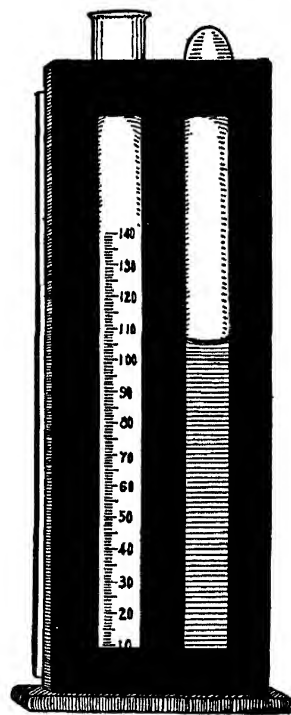


FIG. 110.—Sahli's hæmometer.

paper, and it is also well to cover their upper ends by another piece of paper, that the reader may not be biased by the height of the column. In certain instruments there is a tube for daylight and one for artificial light.

The instrument is not claimed to be accurate within less than about 5 per cent. In buying this instrument it is very essential to get one made by a responsible firm, for the market has simply been flooded by cheap instruments which have not a semblance of accuracy. We recommend those which bear Sahli's name, since he guarantees their

accuracy. It should be remembered that these color-tubes of gelatin stained with picrocarmine certainly bleach, and should be renewed from time to time. When not in use they should be protected from sunlight. An advantage of these instruments is that no greater accuracy is claimed than they possess, and one is not tempted to read to 1 per cent. Another advantage is that there is but one color to standardize instead of a whole scale.

**The Hæmometer of Sahli** (see Fig. 110).—This instrument has been sufficiently tested and certainly is one of the best. The principle of this instrument is similar to that of the Gowers, but the color-tube contains a 1 per cent. solution of acid hæmatin, and the hæmoglobin of the blood is changed to acid hæmatin by hydrochloric acid. Acid hæmatin is a pigment which is quite constant in composition and color value. The instrument may be used in any light since the two tubes contain the same substance and would therefore be modified equally. The blood is obtained in the same way as for the Gowers, and blown and washed into the graduated test-tube into which has already been placed up to the 10 per cent. point a tenth-normal solution of hydrochloric acid. (This may be made with sufficient accuracy by diluting 15 cc. of the pure acid to one litre with distilled water. Sahli recommends that a little chloroform be kept in this stock bottle.) The hydrochloric acid in a few minutes changes the hæmoglobin to acid hæmatin. It is then diluted with distilled water until its tint corresponds to that of the standard color-tube. These tubes are placed in a very convenient little stand with a ground glass back, which renders the reading easy and quite accurate. The color-tube is said not to deteriorate with age so much as that of the Gowers instrument.

**Dare's Hæmoglobinometer.**—This instrument (see Fig. 111), recently put on the market, certainly promised very good results. A film of undiluted blood is compared with a color-prism stained with golden purple. The pipette (see Fig. 112) consists of two plates of glass, one white, A, one clear, B, between which is a slit of known width. A rather large drop of blood is necessary and will at once by capillarity fill the slit. The pipette is then slipped into the instrument, Fig. 111, B, and the reading made at once by the light of a candle, E, attached to the instrument, not necessarily, however, in a dark room, providing the observer faces a black background. On the instrument is a telescope tube, A, which allows accurate focussing and also an advantageous magnification of the two color-fields,—that of the blood and of the color-prism. By means of a small wheel, D, the prism is rotated until the colors match, and then the reading is made at the knife edge on the edge of the disk. The same precaution of not tiring the eyes is to be observed in this as in all other color-tests. The advantages of the instrument are that undiluted blood is used; that

it is rapid; that leucocytes do not affect the reading as in the other instruments; and that it can be used in a light room. Its disadvantages are that certain of the instruments were not well standardized when put on the market, and while they may now be, still the readings are generally rather low; the instrument is expensive; the readings must be made at once before clotting, since in a very few minutes the reading jumps from 5 to 10 per cent. It is rather a fragile instrument and does not stand the wear and tear of hard usage. Nevertheless, it is rapidly cleaned, it is a very convenient, satisfactory, and, when well standardized, accurate instrument. We do not recommend it as superior to the Miescher, and we do not think it enough

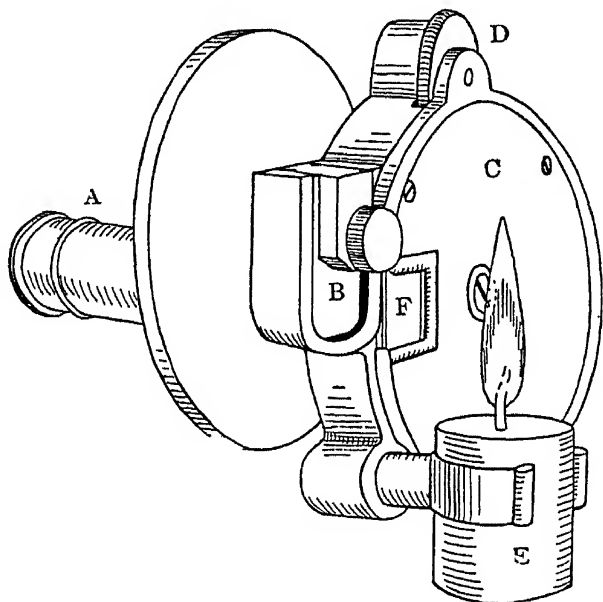


FIG. 111.—Dare's hæmoglobinometer. A, telescope; B, pipette in place; C, case inclosing color-prism; D, milled head moving prism; E, candle; F, window admitting light to color-prism.

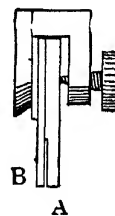


FIG. 112.—Pipette of Dare's instrument. A, the white glass; B, clear glass disk.

better than the cheaper instruments, especially the new Sahli, to justify its high price. We have been very much pleased, putting several instruments in the hands of as many workers, to find how closely they agree in their readings on the same case.

**The Oliver Instrument.**—This instrument is historically important, since it was the first accurate instrument proposed to replace the v. Fleischl, which before then had been accepted as the standard. It consists of two frames of tinted glasses, six disks in each, the colors of these glass disks varying by 10 per cent. The blood is obtained in a large automatically filling pipette, is mixed in a cell with white base, and covered with a cover-glass. By candle-light the color of this mixture is compared with the various glasses until it is decided between which two it stands. The eye must be screened by means of a camera lucida from side rays. By means of riders of colored glass, which raise the percentage 5 per cent. (certain special instruments have a rider for each per cent.), closer readings may be made. The instrument is well standardized and well made. While formerly

it was more accurate than any on the market, it has lately been displaced by the Miescher. Many find it is rather inconvenient to use.

**The Tallqvist Scale.**—This simple little device was exploited as a great boon to the general practitioner. It is a little book of blotting-paper and a scale of colors. A drop of blood on the blotting-paper is in direct sunlight matched against this color-scale. The instrument costs but a dollar and a half, can be carried easily in the pocket, and a determination made in less than a minute. The colors of the scale vary by 10 per cent., therefore any intermediate percentage must be estimated by the eye. We admit that the trained eye, that is, an eye trained to use more accurate instruments like the Miescher, will soon use this color-scale more accurately than an untrained eye will use the Miescher; but the former can guess as near, even from the color of the lips, without the Tallqvist scale, while of one eminent Viennese it is said that he could guess within 0.5 per cent. from a stain on cloth. This was guess-work, but almost as much so is the Tallqvist method. The instrument was standardized against the v. Fleischl, which does not recommend it.

The spot of blood is obtained by holding the edge of the paper against a large drop which soaks up into the paper. This is then blotted by squeezing it between two pages of the book until the lustre is lost, and the reading is made at once before the drop becomes dry. In comparing the tints, position is of great importance. The observer, facing the sunlight, holds the book rather low before him, so that light is well reflected from the color-scale. A moist unstained ring around the drop is seen in anæmia. Leukæmic blood is poorly absorbed, and has a color not agreeing with the scale. So great has been the demand for these books, following their recommendation in a very excellent text-book, that many editions have been published. There is now a slight reaction setting in against this scale. The personal element enters in too largely, as two persons can differ widely in their opinion, and the trained eye sometimes even judges the tints of the scale, and reads without it. We hope the reaction will lead the practitioners to adopt some instrument requiring less guess-work.

In hospital work we find it a great advantage to have several varieties of hæmoglobinometers in use, and to insist that the student shall use them all. Only in this way will he appreciate the strong and weak points of each. The man who uses but one soon places undue reliance upon its accuracy. Let him use two of different makes and get differences of from 5 to 10 per cent., and he appreciates the need of a standardized instrument.

We have one, a new Miescher, which we reserve as the standard. When a clinical clerk signs for an instrument for ward work, he must first standardize the instrument against this Miescher, and in future work make the necessary correction. Among our forty instruments of seven different types we find that the corrections necessary vary from 1 to 10 per cent., and are especially large in those over two or three years of age.

These instruments very seldom read 100 per cent. in a normal person. From the records of 176 medical students, all normal men during the third decade of life, the readings made with great care, the Fleischl instruments in 161 cases read from 65 to 110 per cent.; 136 varied from 80 to 100 per cent., and 52 from 90 to 95 per cent.; the mean was 92.5 per cent. Of 156 records with the Dare instrument, varying from 65 to 110 per cent., the blood of 105 read from 90 to 100 per cent.; the mean 95 per cent. Of 150 students using the Gowers, the records varied from 70 to 120 per cent.; 81 stood between 90 and 100 per cent.; the mean about 92 per cent. Here the direct readings of the instruments not corrected with the Miescher are given.

With the Miescher the blood of 125 students varied from 11.4 to 17.6 gms. per 100 cc. (The estimations were controlled for the most part by determinations at the same hour of the following day.) It is much harder or even impossible to state the mean, so uniform was the distribution. The average was about 14.5 gms. Considering this 100 per cent., the blood of 38 students varied from 90 to 100 per cent., and 21 from 100 to 105 per cent. (Note how different the readings on the other instruments.) The variations with the Miescher seemed great, yet they ran more parallel to the blood-counts than did the others. (See page 531.)

In conclusion, the Miescher is a laboratory instrument, and in clinical work is reserved for special cases; then it alone should be used. For the general run of cases without hæmatological interest, either the Sahli, Gowers, Dare, Oliver, or Fleischl is good enough, provided it be a standardized instrument, for it is the scale which is incorrect, and becomes more so with time.

**Jolles Ferrometer.**—This method, proposed as the most accurate way of determining the amount of hæmoglobin, determines the absolute amount of iron present in the blood. We shall merely outline the method. A small amount of blood is incinerated, the iron of the ash dissolved in a little acid potassium sulphate, and the solution of iron then estimated colorimetrically by means of the ferrometer. In this apparatus the blood-iron solution is placed in one tube, in another tube a standard iron solution. The fluid from this latter tube is allowed to escape through a stopcock, drop by drop, until the tint of the two solutions is the same. For this method a small amount of blood, 0.05 cc., is all that is necessary. The incineration is done in a platinum crucible, the blood being first dried, then incinerated, the potassium sulphate, 0.1 gm., added, and the mixture carefully fused. After it is cooled the ash is then washed into the standard tube. The fluid in the tube used for comparison contains 0.5 mg. of iron and 0.1 gm. of the acid potassium sulphate per cubic millimetre. To each cylinder are then added 1 cc. of dilute hydrochloric acid (1 to 3 of water) and 4 cc. of ammonia sulphocyanide (7.5 gms. per litre). With the apparatus is a table giving the values in hæmoglobin for the various readings.

This instrument is not in use now, for various reasons. In the first place, the hæmoglobinometers are more accurate than they were at the time when this was used. In the second place, it was found that the results with this did not agree well with those of the hæmoglobinometers, and hence a different chemical composition of the hæmoglobin was assumed, or the presence of iron in the blood in other organic compounds. Even this was named "hæmatogen," or hæmatinogen. Both of these arguments are rather lame, since hæmoglobin is a rather constant body, and in the very small amount of blood used the amount of iron in other forms would hardly produce an appreciable error. Perhaps it is the method itself which is at fault. The method has been severely criticised by Pekar,<sup>35</sup> who describes a Winkler method, another quantitative determination of the iron.

<sup>35</sup> Maly's Jahresbericht, vol. xxxiii. p. 241.

Rosin and Jellinek,<sup>37</sup> comparing the Jolles instrument with a Miescher hæmoglobinometer, find no fixed relation between hæmoglobin and total iron. Some cases have high color, diminished iron, a variable count, as uncompensated heart disease. Some have high color, low iron, and normal count, as jaundice, diabetes, Graves's disease. Some have low color and high iron, as some anæmias and chlorosis.<sup>38</sup>

**Hæmoglobin.**—One hundred cc. of normal blood are usually stated to contain from 13 to 14 gms. of hæmoglobin. Careful estimations of the hæmoglobin at the various ages have shown a regular age curve, quite parallel to that of the red blood-cells, but with variations a little more pronounced.

Age.	Gms. per 100 cc. of Blood.
1 to 4 days .....	19.329 to 21.160
8 to 14 days .....	17.869 to 16.124
8 to 20 weeks .....	15.362 to 12.928
6 months to 5 years .....	10.971 to 11.373
5 to 15 years .....	11.151 to 11.796
15 to 25 years .....	13.034 to 13.870
25 to 45 years .....	14.727 to 15.013
45 to 60 years .....	12.484 to 13.150

From this table of Leichtenstern (modified from Sahli's quotation) it is at once evident that the age curve must be considered in all blood-work, and the hæmoglobin given in grammes per 100 cc. rather than in percentage, since there is no one figure which could be considered 100 per cent. for all ages.

By *oligochromæmia* is meant a relative diminution in the amount of hæmoglobin per unit volume of blood. It therefore is seen to have merely a relative and not an absolute value.

By *color index* is meant the percentage of hæmoglobin divided by the percentage of the red blood-cells. This figure, as Duncan first showed, is of considerable importance in some cases. For estimating the denominator, 5,000,000 red blood-corpuscles are considered 100 per cent., while the numerator is the per cent. of hæmoglobin read with any instrument. The color index is less than 1 in all cases in which the blood after anæmia is regenerating, hence in all secondary anæmias, and especially in chlorosis, in which the average is about 0.5, in some cases being as low as 0.3. In pernicious anæmia, on the other hand, the index is increased, the average in a large number of cases of Cabot being 1.04, and one case reaching 1.75 (count 1,000,000, hæmoglobin 35). The high color index is of especial value in differentiating pernicious anæmia from certain cases of cancer of the stomach, a diagnosis clinically hard to make. To be of value, how-

<sup>37</sup> Zeits. f. klin. Med., Bd. 39, p. 109.

<sup>38</sup> See also Mayer, Zeit. f. klin. Med., 1903, vol. xlix. p. 475.

ever, the variations must not be in the second decimal place. It is not a strict mathematical calculation; the figures are too approximate for that.

The question arises at once, What is the color index reckoned in the above manner for the average normal man and woman? Five million is only in very general terms the normal figure, and few hæmoglobinometers read normal blood at 100 per cent. As stated on page 499, taking instruments as they come, the index in fairly normal persons varies from 0.80 to 0.88. In the case of our students, the men, with the Fleischl and Gowers instruments had an index of 0.84, with the Dare, 0.87; the women, with the Fleischl, 0.88, Dare, 0.9, Gowers, 0.82. Yet we often see cases with the above indexes reported as "mild chlorotic anæmia." All our cases are to be judged in the future with this normal index in view.

The question was approached in still another way. Our students' work included fifty-three records of counts and Miescher hæmoglobin estimations made on the same bloods at the same hour. The counts varied from 4,600,000 to 6,700,000, and the hæmoglobin from 10.9 to 17.2 gms. If in each case the number of grammes of hæmoglobin per 1,000,000 cells be reckoned, the mean of these figures should be an approximately normal color-index. These quotients fell within surprisingly close limits, 42 of the 53 varying from 2.2 to 2.8 gms.; mean, 2.63 gms. per 1,000,000 cells. Using this figure as the standard of hæmoglobin content, we hope in the future to be more accurate in our use of the color index.

One point we may be pardoned if we emphasize. Students seem to consider that the mechanism regulating the blood-count is almost as delicate as that controlling the body heat, and that the count of the blood of normal men should vary almost as little as does the temperature, although that varies somewhat. The more carefully the counts are made, the better the instruments used for hæmoglobin, the more evident are the individual, both general and local, the daily and seasonal, and the racial, variations. It is more like the height, weight, or muscular development. And yet the regulation of the composition of the blood is wonderful. It varies within quite narrow limits, although through the vessels pass enormous amounts of water as in diabetes insipidus, of water and solids as in diabetes mellitus, of albumin and water, as is seen in cases of rapidly collecting ascites repeatedly tapped with the frequent withdrawal of even 8 litres of what is practically blood-plasma, while the blood remains wonderfully little changed. Yet no one figure is normal for all men, nor constantly for one man. The same is true of the total amount of hæmoglobin per 100 cc., and also for that per cell. Hence, in judging of blood reports one must not try to apply any hard or fast rules, but to study these variations, since they may be put to practical use.

The "*volume index*," or the quotient of the volume per cent. and the count per cent. ( $5,000,000 = 100$  per cent.) promises well.<sup>39</sup> To determine the volume of the corpuscles Capps uses the hæmatocrit and undiluted blood. A length of the column of corpuscles of 50 per cent. he accepts as normal, hence the 100 per cent. of the calculation (see page 471).

The most important result he obtained is that in pernicious anæmia the color index never exceeds the volume index; that is, that there is no supersaturation of corpuscles with hæmoglobin, and the high color index is due to increase of size alone. Yet on the other hand, in other anæmias the color index may fall below the volume index, and the color index drops more rapidly than the volume, while

<sup>39</sup> Capps, Jour. of Med. Research, 1903, vol. v.

during regeneration the volume returns to normal first. He has never seen any evidence of "acute dropsy" of the cells.

#### WHITE BLOOD-CELLS

**Granulations of Leucocytes.**—By granules are here meant the minute bodies, usually spherical, of a size and staining character fairly constant for each granulation, which seem to be not accidental but to belong to the protoplasm; they are perhaps the product of the secretory activity of the cell, but not in the sense that all protoplasm is slightly and indefinitely granular; they are definite inclusions in the cell protoplasm, and are liberated as independent bodies which swim free in the plasma when the cell breaks up. From them can perhaps be distinguished degeneration of protoplasm, accumulations of products of metabolism, and inclusions from phagocytosis.

The various granulations as classified by Ehrlich are, **EOSINOPHILIC**, **ACIDOPHILIC**, or **OXYPHILIC** ( $\alpha$ ). These granules are coarse, about 1 micron in diameter, spherical or slightly oval, quite uniform in size and color, are very refractive and so in the fresh specimen appear black. From a mixture of stains these will always take the acid ingredient. These granules have been found in the blood of every animal whose blood has been examined, from the frog to man. They are of albuminous nature and not fatty as their appearance would indicate. Barker has found that they contain iron.

**AMPHOPHILIC** ( $\beta$ ).—These granules are said to vary in size, some to be as large as  $\alpha$  granules, others considerably smaller. Some are said to take acid and others basic dyes. They stain like the eosinophilic granules, except that in a mixture of eosin and indulin they will take the latter; they also take certain basic stains. They occur often in the same cell as the  $\alpha$  granules, hence Ehrlich considers them a younger stage of these, and this to be the only case in which two specific granulations are found in one cell. They are found in some leucocytes of the bone-marrow of man and various animals (rabbit and guinea-pig), and in the peripheral blood in certain anæmias. These may explain, in leukæmia for instance, the variations in the size and tint of the granules in some of the eosinophile cells. A few may be present in an eosinophile cell.

**BASOPHILIC AND MASTZELL GRANULES** ( $\gamma$ ).—In the connective tissue and blood of all animals and man are cells which contain large basophile granules. Those of the tissues stain best with dahlia, taking a metachromatic tone rather than strictly basophilic, resembling that of mucin, of which, indeed, some claim that they are composed. This tone is best seen if polychrome methylene blue be used, and is explained as due to the methylene azure of this stain. The granules are spherical or oval in shape, and vary considerably in size in the same cell. Cells



containing somewhat similar granules occur to a small per cent. in normal blood, and are increased in leukæmia, while in some cases of pleural exudate and of gonorrhœa this may be the chief granulation of the leucocytes of the pus. Considerable doubt exists, and is admitted even by Ehrlich, concerning a relationship between the basophile cells of the blood and of the tissues, the latter the true Mastzellen. Their origin seems different. In fact, their only resemblance seems to be that both have basophile granules, yet which do not stain exactly alike. What is more, considerable doubt exists whether the  $\gamma$  granules in cells of abnormal blood are exactly the same as those present in the normal blood and bone-marrow, since even between these certain variations in the staining qualities exist, and those in leukæmic blood are more soluble in aqueous solutions than those of normal blood. It is, therefore, at least possible that we have here to do not with one specific granulation, but with three or more different granulations, or with the same granulation at different stages of its development.

**BASOPHILE GRANULATIONS ( $\delta$ ).**—These granules were originally described by Ehrlich as occurring in the mononuclear cells, especially the lymphocytes; as not staining by dahlia, and hence differed from  $\gamma$  granules; and occurring especially in the cells of bone-marrow. Ehrlich, while he has not publicly repudiated these, leads one to suppose that he now considers them to be not true granules, but nodes of the reticulum of the protoplasm.

**NEUTROPHILE GRANULES ( $\epsilon$ ).**—While a somewhat similar granulation occurs in some animals, cattle, swine, and sheep (Hirschfeld), neutrophile granules of this size, arrangement, and color are found only in man, and hence are considered by Ehrlich specific for him. They are extremely fine, dust-like, and occur in the mononuclear cells of the bone-marrow, a few in transitional cells, and fill the ordinary finely granular cells of the blood. With the Ehrlich triple stain they take a lilac color, and this is really the only specific stain for them, but they also will take an acid stain; hence others name them the “fine oxyphilic granulation,” in contradistinction to the eosinophilic or “coarsely oxyphilic.”

**NEUSSER'S PERINUCLEAR GRANULATION.**—In certain leucocytes, mononuclears especially, and polymorphonuclears, but in some specimens stained with Ehrlich triple stain in any of the leucocytes, are sometimes seen blackish-green granules, which always appear attached to the nucleus. Their size varies much, and they have often a glistening or refractile appearance. Neusser considered them as characteristic of the uric acid diathesis. It has been shown since then<sup>40</sup> that these are in reality artefacts which can be produced by variations in

<sup>40</sup> Fletcher, Centralbl. f. innere Med., 1899.

heating and in the stain, and, indeed, with some mixtures may be produced at will, and which bear no relation to the output of alloxuric bodies in the urine.

**THE GRANULATION OF LYMPHOCYTES.**—In well-spread specimens stained by the various modifications of the Romanowski stain are seen fine violet granules in about one-third of the lymphocytes, especially those with a fairly wide protoplasmic margin. They are not always spherical; their size is between the  $\alpha$  and  $\epsilon$ ; few or many may be present in one cell, yet, as a rule, they are not too numerous to count. They occur also in the large mononuclears and transitionals. They cannot be stained by the Ehrlich stain. By this discovery Michaelis and Wolff<sup>41</sup> consider that they have broken down the sharp line of demarcation drawn by Ehrlich between the granular and the non-granular cells. They are not found in cells from smears of lymph-glands, or of marrow. Ehrlich replied that while it cannot be denied that these were granules, yet they cannot be considered as forming a definite granulation in the sense in which he used the term, since they varied so in number in the type of cells containing them; nor did they occur in all the cells of the class in which some were found; and they required a very special method of staining to demonstrate them.

**FATTY GRANULES** occur in the leucocytes in cases of hyperpyrexia, are easily recognized and easily stained with Sudan III.

Ehrlich's classification of granules is exceedingly satisfactory as a text-book description, but one working much with the blood finds that nature has not drawn the lines so sharply. Ehrlich admits that both  $\alpha$  and  $\epsilon$  granules develop from basophile granules. In eosinophile cells there may be  $\alpha$  and  $\beta$  granules; apart from this possibility, all  $\alpha$  granules are not of the same size, but some larger ones are mixed in, especially in the cells of bone-marrow, sometimes a few in one eosinophile, sometimes many. These may resemble myelin or other degenerations or cell inclusions, and, indeed, the larger or most may be, but excluding these, even in well-stained specimens the size is not always uniform. The view is to be mentioned that all eosinophile granules are the results of phagocytosis of fragments of red cells, or of platelets. Of the  $\gamma$  granules, at least two varieties exist and perhaps three. Cells with various sizes of  $\epsilon$  granules occur, and artists employed to illustrate articles on blood absolutely refuse to picture them all of the same size. Again, the line between the  $\alpha$  and the  $\epsilon$  granules is not always sharp. It may be an individual peculiarity, but in the blood of some patients occurs a large group of cells with acidophilic granules, which one hesitates to classify as neutrophile cells, since they are so large, or as eosinophiles, than which granules they are slightly smaller. This is true in fresh preparations as well as in the stained specimens, and has been admitted by several observers, especially in cases of trichinosis, hence some consider them transitionals (Brown, McCrae, Anderson). And, lastly, especially in smears of the bone-marrow and leukæmic blood, the line between the granular and non-granular cells is exceedingly difficult to draw, since there are so many mononuclear cells with a protoplasm suggesting a faint granulation.

<sup>41</sup> Virch. Arch., Bd. 167, p. 151.

We do not overlook the fact that much depends on technic. Of a set of smears made from the same patient at the same time, but heated and stained with slightly different technic, the granules will appear of somewhat different size and of markedly different color-tone. It is with due allowance made for this that we make the above statements.

**Leucocytes.**—We give, first, *Ehrlich's classification*, since that is the one in common use.

**LYMPHOCYTES** (Plate I., 1, also 3 and 4).—These cells are smaller (5 to 8 microns in diameter) or larger (8 to 10 microns) than the red blood-cells. The nuclei are relatively large, round, quite deeply stained, centrally placed as a rule, and have one or two nucleoli. They may be deeply notched, especially the smaller ones, and even suggest a polymorphonuclear cell, but are never just like it. Often there is a clear band between nucleus and protoplasm. The protoplasm forms a narrow rim around the nucleus, is sometimes acidophilic (older cells?), but generally basophilic, often more so than the nucleus, and takes a grayish-green stain with the triple stain. Of other cells the nucleus seems naked. The larger cells of this group have an irregularly staining nucleus with a chromatin network and a faintly granular margin of protoplasm. These latter forms may be exceedingly large in lymphatic leukæmia and in the blood of normal infants. It is rare to see them in other conditions. These cells, if stained with the polychrome methylene blue-eosin stains, show a distinct granulation in about one-half their number.

The lymphocytes constitute from 22 to 25 per cent. of the leucocytes in the normal adult blood, and from 40 to 60 per cent. in the infant's.

**LARGE MONONUCLEARS.**—By "mononuclear" is meant that the nucleus is round or lobulated but not polymorphous. These cells are two or three times as large as red blood-cells, have a large, oval, vesicular, eccentrically placed, faintly staining nucleus, which indeed may be overlooked, and abundant weakly basophilic protoplasm without granules (Ehrlich stain). Nodal thickenings are present, and by Nocht stain some show a granulation. These cells constitute about 1 per cent. of the leucocytes of the normal adult blood. While in normal blood these cells are practically all large, in other conditions, especially typhoid fever and malaria, this group may be represented by all sizes from that of lymphocytes to large giant-cells. The small forms it is easy to distinguish from lymphocytes, but they occur very seldom in normal blood. (Plate II. The group 9–20 contains many.)

**TRANSITIONAL CELLS** (Plate I., 5).—These cells resemble the large mononuclears, but are often larger,—in fact, the largest cell of the blood. The nucleus is much notched, giving the so-called "wallet" or "saddle-bag" nucleus. The protoplasm stains quite deeply in Ehr-

lich's stain, and often presents a very few neutrophile granules. These constitute from 1 to 3 per cent. of the leucocytes of the normal adult.

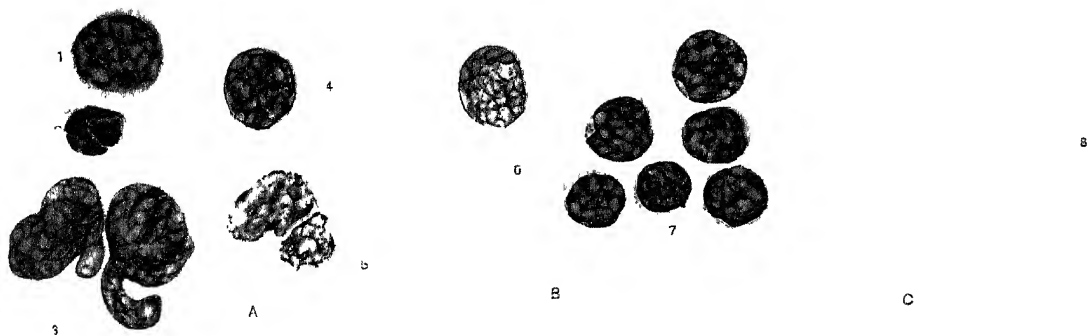
**POLYMORPHONUCLEAR NEUTROPHILES** (Plate I., 6, 7).—These cells, which constitute from 70 to 72 per cent. of the leucocytes of the adult and from 18 to 40 per cent. of the child's, are somewhat smaller than the transitional cells. When spherical they are about 10 microns in diameter, but in a well-spread smear, in which they have flattened out upon the glass, they may seem about twice this size. The amount which they have flattened explains their varying size, so often deceptive in smears of unequal thickness. The nucleus is characterized by its polymorphous nature and its deep stain, due in many cells to pycnosis. It may be a strand variously bent, or small fragments, two or more in number, connected by fine filaments. The protoplasm takes a faint acid stain. It is well filled with the neutrophile granules which may cover the nucleus. When migrated these are the ordinary pus-cells. They contain glycogen in certain conditions.

**EOSINOPHILES** (Plate I., 2).—These cells are of the same size or perhaps a little larger than the preceding. The nuclei may have the same shape, yet less pycnotic, fainter staining ones are the rule. The protoplasm is often slightly more abundant, and is filled with eosinophilic granules, which do not often lie upon the nucleus. These cells constitute from 2 to 4 per cent. of the normal leucocyte count.

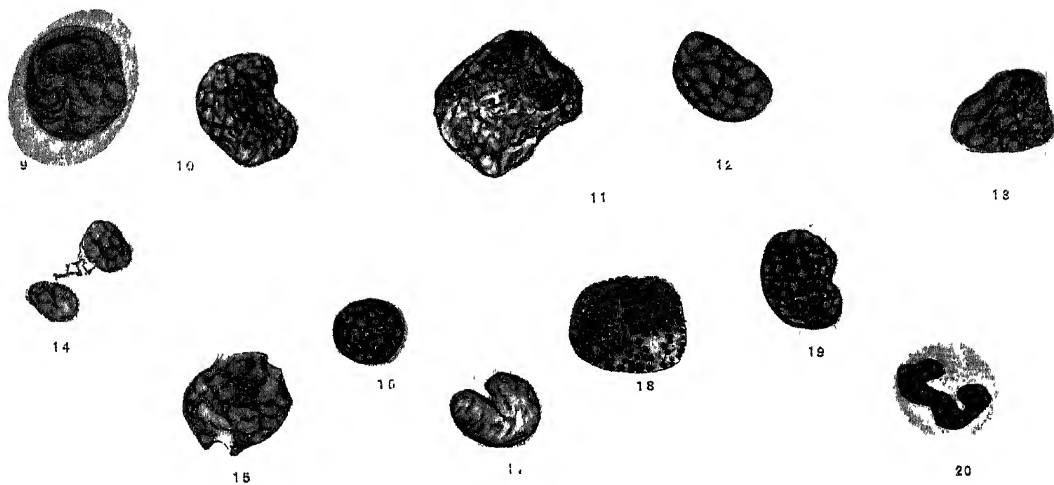
**MASTZELLEN** (Plate I., 8).—This name is given, perhaps incorrectly, to any cell with basophilic granules. It is not at all certain that these are in any way related to true Mastzellen of the connective tissues. These cells are about the same size as the preceding, but more often smaller. The nucleus is polymorphous, very faintly staining, often trilobed. The protoplasm contains a variable number of granules of different sizes, yet for the most part as large as  $\alpha$  granules, which form a band around the nucleus. These granules are not stained by the triple stain, hence one sees only the spaces occupied by them. (These are probably the reticulated or vacuolated cells of Uskow.) They stain best in thionin and are said to take a metachromatic tone. These cells constitute about 0.5 per cent. of the total count. They have every appearance of old cells: are small, do not spread well, but look shrivelled, with acidophilic protoplasm and polymorphous nucleus.

The leucocytes found in pathological conditions are:

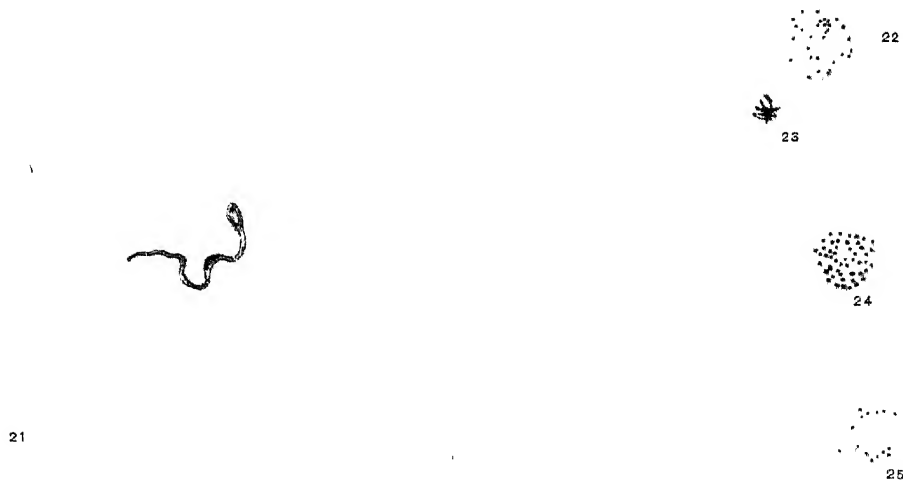
**MYELOCYTES** (Plate I., 9, 11, 14, 17).—While any cell of bone-marrow is, strictly speaking, a myelocyte, by this term is generally meant one with a round nucleus and granular protoplasm. Neutrophilic and eosinophilic myelocytes occur. Their size varies from that of the large mononuclears to that of red corpuscles. The largest and smallest neutrophile myelocytes are found in the blood only in



LYMPHATIC LEUKAEMIA.



LEUCOCYTES OF NORMAL BLOOD AND MALARIA



TRYPANOSOMA GAMBIENSE,  
FROM A CASE OF "SLEEPING SICKNESS"  
STAINED WITH HASTING'S MODIFICATION OF  
ROMANOWSKI'S STAIN. ALL DRAWN TO SAME SCALE

BASOPHILE GRANULES  
OF RED BLOOD CELLS.

F. S. Lockwood.



myelogenous leukæmia, but a few, the size of the granular leucocytes may be found in any condition with a high leucocyte count. The characteristic point is the shape of the nucleus, which is either perfectly round, oval, indented or kidney-shaped, but never polymorphous or pycnotic; if it were, the cell would count as an ordinary leucocyte. It is usually centrally placed. It is impossible to draw a sharp line between a myelocyte and polymorphonuclear cell (Plate I., 12, 13), since every possible gradation occurs. But one soon forms a standard for himself. As myelocytes we count all cells with round, oval, or kidney-shaped nuclei, providing the nucleus occupies at least one-half of the cell. Such a nucleus will not be stained diffusely by any good nuclear dye. A cell with nucleus more compact, more distorted, or more diffusely stained than this ranks as a leucocyte. The specification "round or oval nucleus" needs a further qualification, for one sometimes sees such nuclei in leucocytes. But in leucocytes they are relatively small (occupying only about a quarter of the diameter of the cell) and stain diffusely. One is tempted to believe that could he get a side-view of them, they would be polymorphous. Their lack of chromatin net-work also is enough to prove them leucocytes. For the question of the motility of myelocytes, and most now agree they are motile, see the writings of Wolff.<sup>42</sup>

Some myelocytes are full of granules, some have but few, and they are scattered. The very large forms occur in the bone-marrow and in well-made specimens of the blood of myelogenous leukæmia, but of the most of them only a large faint nucleus and granules free in the plasma are seen. Some have a large nucleus and a narrow rim of protoplasm, others a smaller nucleus and more protoplasm. Still others are very small.

**EOSINOPHILE MYELOCYTES** (Plate I., 14).—These are the exact analogue of the preceding, and occur in much the same conditions, but less often and in much smaller numbers. They are found especially in splenomyelogenous leukæmia and in anæmia pseudolymphatica infantum.

**SMALL NEUTROPHILES; PSEUDOLYMPHOCYTES.**—These cells have a round, intensely staining nucleus and a narrow margin of protoplasm full of neutrophile granules. Their size is about that of a lymphocyte. They are rare, occurring especially in pleuritic exudates, and are supposed to arise from fragmentation of the polymorphonuclear cells.

**IRRITATION FORMS.**—The description given of these cells is the following: They vary in size from a lymphocyte to a large mononuclear, but resemble the former more; the nucleus is round, of a bluish-green color (Ehrlich stain), often eccentric and without a chromatin net-work; the protoplasm stains an intense rich brown; these have no granules. Türk considers that they have the same occurrence and meaning as myelocytes.

<sup>42</sup> Deut. med. Wochenschr., March 5, 1903.

*Uskow's Classification.*—This writer has given a classification of leucocytes based on specimens stained with the triple stain, more objective than that of Ehrlich, and although not in common use, yet valuable to bear in mind when using any classification.

A. *Lymphocytes.*—Cells with a round nucleus, narrow ring-like rim of protoplasm separated from the nucleus by a clear sharp circle, the nucleus and protoplasm staining intensely.

(1) *Small Lymphocytes.*—The size of red blood-cells or smaller, with a uniform ring of protoplasm.

(2) *Lymphocytes.*—A little larger than red blood-cells, the rim of protoplasm often thicker on one side, giving the seal-ring appearance, or with two or three prominences.

B. *Transparent Corpuscles.*—Characterized by the quantity of protoplasm which with the triple stain remains colorless. The nucleus is homogeneous, round, oval, or bean-shaped, usually eccentric and stains a feeble pinkish shade.

(3) *Small transparents,* size of large lymphocytes, or a little larger, often in the form of a square with rounded corners.

(4) *Large,* three to five times that of a red cell; the nucleus eccentric.

(5) *Lobulated Forms*—These are the largest cells of the blood. The nucleus is on one side, usually toward the centre of the corpuscle, with one or two deep indentations.

C. *Transitional Cells.*—These have many of the characteristics of lymphocytes and transparents. They are rich in a protoplasm which is sometimes slightly granular and always takes a good stain, but less intensely than that of lymphocytes. The nucleus stains more deeply than the protoplasm and usually has no clear ring around it.

(6) *Small Transitionals.*—Resemble giant lymphocytes or colored small transparents.

(7) *Transitionals,* are larger.

(8) *Lobulated forms,* the largest of this group.

D. *Polymorphonuclears.*—The nucleus intensely staining and of various shapes. Protoplasm abundant, coarsely or finely granular.

(9) With thick, rod-like nucleus, which stains rather feebly. Granules smaller than those of the other neutrophiles.

(10) Nucleus like a bent rod, often twisted at one end.

(11) Multinuclear, the fragments, however, connected by strands of chromatin.

Uskow considered the above classification based on age. As young forms, he considered the small and large lymphocytes and the small transparents; the ripe elements were all the transitionals and the large and the lobulated transparents, while the over-ripe forms have polymorphous nuclei. The classification is based on Ehrlich's stain, and hence cannot, as a whole, be as well applied to specimens otherwise stained. It is, however objective, and gives a much better description of the cells actually found than does the Ehrlich.

**Differential Counting.**—For a differential count a satisfactory classification is a necessity. Since we know so little of the relationship between the various leucocytes, of their age, their changes, their origin, and of their function, the only classification possible would be a purely morphological one. Pappenheim has proposed such a one, but his is too complicated for clinical use, hence Ehrlich's, being the simplest, still obtains. Ehrlich separates in the normal blood, small mononuclears, large mononuclears, transitionals, polymorphonuclear neutrophiles, eosinophiles, and Mastzellen.

By small mononuclear is meant any non-granular cell smaller than a polymorphonuclear neutrophile. This group would include, there-



fore, all lymphocytes and the small transitionals and transparents of Uskow. As large mononuclears are classified any non-granular cells larger than a polymorphonuclear neutrophile, with a round or oval nucleus; any cell within the same size limits, but with an indented nucleus, is called a transitional. The polymorphonuclears, both neutrophiles and eosinophiles, are clear enough. As a Mastzell, is counted any polymorphonuclear cell without granules (Ehrlich's stain), or with blue granules if methylene blue is used.

For normal blood this classification is satisfactory, but for pathological conditions many objections arise. While the lymphocytes seem to form one distinct class (Plate I., 3, 4, 15, 20), the other mononuclear cells it is impossible to classify on the staining character of their protoplasm, but this point is quite certain, the group of large mononuclears has large, medium, and small forms, and any line dividing this group is purely arbitrary; it increases the number of the lymphocytes by cells which do not belong there, and diminishes a group which should not be divided. This is best seen in typhoid fever and malaria, in which diseases the group of the transparent cells of Uskow is increased as a whole, both the large and small forms; and in the so-called lymphatic leukæmia, in many cases of which the mononuclear cells are certainly not lymphocytes.

Neither is it just to separate groups on the basis of an indentation of the nucleus. Ehrlich's name "transitional" still exists, but as soon as, by means of the triple stain, he discovered the myelocyte, he saw at once that the transitionals were no longer necessarily a step in the development of a polymorphonuclear cell. It is now generally agreed that these large cells with indented nuclei are only older (senile) forms of those with oval nuclei, and need no longer be counted as separate. As regards the transparents and transitionals of Uskow, it may well be, as some suggest, that the younger cells have a more basophilic protoplasm, while the older cells have an acidophilic; or these cells may be of really distinct origin.

The line between myelocyte and leucocyte is very hard to draw, since no line can exist in an unbroken series of intermediate forms.

In leukæmia to draw a line between large mononuclears (Plate I, 16, 19, 21) with deep staining protoplasm and granular myelocytes is also very hard, since perhaps here also no line exists; yet in well stained specimens one is in doubt concerning but few cells.

One group of cells does confuse us, especially in heated specimens, cells which are represented merely by a faintly staining nucleus, and that this is a nucleus one is often in doubt; especially if one control his count by one on the fresh blood, then he is sure all are not leucocytes. Such cells should not be passed over, but counted as "undetermined cells," for only in that way will the percentage of those groups which are more resistant be fairly correct. These undetermined cells are almost all large mononuclears, yet if many are found on preliminary examination of a smear, that smear should not be used for a differential count.

To differentiate eosinophiles and neutrophiles there should be little difficulty in a well-stained specimen, and yet in certain cases the question is very hard, and one doubts for that case the specificity of granules. We believe that most observers do not pay much attention to a point formerly emphasized, the characteristic lilac tint of the neutrophile granules; that they do not believe that true eosinophile granules may be as fine as neutrophiles and distinguished by their color-tone alone, as do some who have written concerning eosinophilia. To most of us neutrophile granules are fine and eosinophile coarse, and little attention is paid to their color except as a basis to criticise the staining mixture used. One further point is important. It is customary to count neutrophile myelocytes in a class by themselves, but eosinophile myelocytes with the eosinophile leucocytes. We are not at all sure this is a fair method.

For differential counting we generally use specimens stained with Ehrlich's triple stain, but routine clinical work is done more easily and probably as accurately (some say more so. Barnes) with specimens stained with Hastings's stain. Of the non-granular cells one makes two classes, small and large mononuclears, although some still prefer to recognize Ehrlich's transitional cells, and others, following Uskow, distinguish the transparent and transitional cells. Yet in routine ward work and for the sake of uniformity we still separate small and large mononuclears, using the polymorphonuclear neutrophile as the size-line, but count large mononuclears and transitionals together. The granular cells are divided as neutrophiles, eosinophiles, and basophiles. Separate classes are made for  $\epsilon$  myelocytes and  $\alpha$  myelocytes. Nucleated reds are also counted and calculated as "number per thousand leucocytes."

The list of cells is, therefore, the following, using the customary abbreviations: S. monos., or s.m.; l. monos., or l.m.; tr.; pmn.  $\epsilon$ , or pmn. n.; pmn.  $\alpha$ , or pmn. eos.; Mastz., myeloc.  $\epsilon$ , myeloc.  $\alpha$ , nucleated reds, normobl., intermed., megalobl.

For a differential count a mechanical stage should be used, and at least five hundred, better one thousand, leucocytes counted. Yet one can get a fair idea of a slide without a mechanical stage. Some keep count with a pencil and paper, the paper ruled, one column for each group; others use a slide-box divided into compartments by slides, into which he drops beans, one bean for a cell. Since one starts always with five hundred or a thousand beans, the mathematics of this calculation are easy.

Many use specimens stained with the various polychrome methylene blue-eosin stains for differential counting, with confidence of their ability to distinguish the various granulated cells, and the added advantage of better-stained nuclei and stained basophilic granules. In ordinary use this is very well (Plates II., III.). The average Ehrlich triple stain mixture in use is a poor fluid, and gives a poorer picture than these. Ehrlich's neutrophilic granulation has not gained quite the clinical importance which he expected, but it is only fair by Ehrlich to use the term "finely granular cells" or "fine acidophilic," in case other stains are used, and reserve the term neutrophilic in connection with his triple stain, for although the color-tone of the two granulations is different when the former stains are used, the result is not so specifically neutrophile, as with the triple stain.

With these stains (Nocht and its modifications) all nuclei stain much better than with the Ehrlich. The protoplasm also stains much better, an intense blue with very beautiful net-work, or a diffuse blue, or a red. The finely granular cells present a diffuse haze of purplish granules, and many of them can be made out easily and

clearly and their tint seen, but the picture is not so beautiful as in an Ehrlich specimen. The eosinophile granules take the eosin. For very careful work it is advisable to count two specimens, one stained with Ehrlich's triple stain for granules, one with hæmatoxylin-eosin for nuclei; by comparison these will correct each other.

We hope that soon much more differential counting will be done with fresh blood preparations. It is rather hard and often inconvenient, but perhaps more accurate than with stained smears, since what one loses in the tint he gains by avoiding artefacts and broken-down cells.

**Bone-Marrow.**—The study of the bone-marrow is a subject of primary importance. In it are found practically every cell which occurs in the blood in almost every condition; that is, a large number of those cells which are unusual in the peripheral blood, and a complete series of transitional forms between different groups; hence this study renders the blood-pictures more intelligible.

The study of fresh marrow is especially important; that of the ribs of young babies, especially of those born prematurely, is best. Fragments removed in operations for empyema are excellent, and autopsy specimens if fresh enough. It is surprising how quickly some of the interesting mononuclear forms, "young" cells, disintegrate. The large form of myelocytes also soon disappear, and in leukæmia the marrow may soon be of very little value, showing only a confused mass of nuclei in a cloud of free granules. A small piece is squeezed in a pair of forceps and a small drop of the exuding marrow picked up on a cover-glass and at once pressed down onto a slide. Spicules of bone must be avoided. Very rapid work is necessary, since the drop dries very rapidly. The marrow may be diluted with salt solution if desired. For stained specimens, the stroke method is the most useful; that is, the marrow is smeared in lines on the cover-glass by drawing this across the end of the bone. The specimen is allowed to dry in the air and then may be fixed and stained just as blood smears. If the marrow is fatty the smears do not turn out well. If, fixed by heat, it is well to remember that it is easier to underheat than it is to overheat, and the easiest method is to place the cover-glass on the copper plate, smear up, at the spheroidal point (that is, the point at which the drop of water does not boil but merely rolls off the plate) for forty-five or more seconds. Such specimens will for the most part have good areas for study, especially at the edges of the thick portions, and a few such fields are all that is desired. Specimens made thin, in the hope that the surface will be uniformly good, are usually failures, for those too thick are better than those too thin if heat and the Ehrlich stain be used.

Bone-marrow varies much. In some places will be found nests

of nucleated reds in enormous numbers; in other places nests of leucocytes, myelocytes, and of intermediate forms. Different parts of the same rib vary, as we have found to be markedly the case in infant marrow. Since the marrow in different bones and in different parts of the same bone varies so, it is impossible from a limited search to say what is the general medullary condition of that case (Grawitz), and this may explain the lack of evident relation between a marrow and a blood picture.

While it is impossible to really count the cells of the marrow, yet differential counts can be made of those found in measured areas.

NUCLEATED RED BLOOD-CELLS.—The term “erythroblast” is used by some to mean a nucleated red blood-cell; by others a colorless ancestor form of these. We use it for any nucleated red cell.

(1) NORMOBLASTS. (a) *Howell's Mature Nucleated Reds* (Plate I., 29).—These are the color of the non-nucleated red blood-corpuscles, with a pycnotic nucleus 3 microns or slightly less in diameter, sharply defined, without a chromatin net-work, dense, homogeneous, structureless (triple stain), of a dense uniform blackish-green color, often vacuolated, hence in the nucleus is often a bright spot in the fresh and an unstained area in the stained specimen. These nuclei are so characteristic that they may be recognized even if not surrounded by protoplasm. They often present amitotic figures, giving rise to rosette forms of two to four or even twelve fragments. Sometimes these fragments are all connected by strands of chromatin (see Plate I., 34, Fig. 113, c). This nucleus is often surrounded by a clear zone which probably represents space left between the contracting protoplasm and nucleus. The nucleus may not occupy this space, but rest upon a margin of the red cell or even at some distance from it. This is explained by Ehrlich by the weight of the nucleus—when the specimen is made the cells are violently thrown into a new position, which centrifugal force throws the nucleus out of the cell. And yet this will not explain all free nuclei, since they are seen in specimens made in various ways and in sections as well. Pappenheim and Israel claim that in leukæmia especially such free nuclei result from the degeneration of the surrounding protoplasm.

From these cells with very pycnotic nuclei, in which can be seen no structure whatever, are all gradations with the chromatin structure more and more evident till we reach (b) *Howell's immature nucleated reds* (Plate I., 30, Fig. 113, a). These are of a little larger size than an ordinary red blood-cell with color perhaps a little paler; the nucleus is slightly larger, with the chromatin fibres radially arranged (in leucocytes it forms a meshwork [Pappenheim]), while clearly seen mitotic figures are not rare. Division of these cells is rapid, requiring but fifteen minutes. In the bone-marrow this is the dominant red cell.

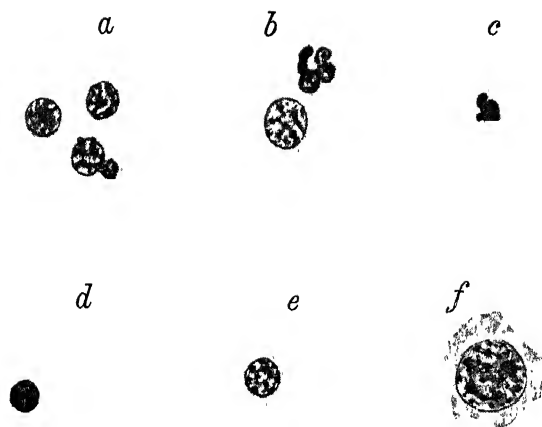


FIG. 113.—Nucleated reds from the blood of a fetus 15 cm. long. *a*, mature nucleated red; *b*, intermediate form and rosette; *c*, mature red, nucleus fragmented; *d*, free nucleus of a mature red; *e*, mature red, polychromatophilic cell; *f*, polychromatophilic megaloblast.



These two forms are generally called normoblasts, although the larger immature forms are by some classed as intermediates. They are the precursors of the ordinary non-nucleated red blood-cell, but do not reach the circulation of a normal adult except as an anomaly. Their appearance in the general circulation indicates an increased activity of the bone-marrow, such as occurs after hemorrhage. They appear in the blood of a child more readily than in that of an adult. Many occur in pernicious anæmia, more in splenomyelogenous leukaemia, and some in post-hemorrhagic anæmias. As in the marrow so in the blood these cells occur in groups often, and lie free from the rouleaux (since heavier than the others?).

These cells are "orthochromatic" normally; that is, they stain like the ordinary non-nucleated reds (oxyphilic). There is a group of fuchsinophilic cells which some consider young forms, but which Engle considers a distinct group; other cells, especially in pernicious anæmia, are polychromatophilic.

"BLOOD CRISES" is the term given by v. Noorden to the periods, usually in the convalescence of an anæmia, during which for a few days enormous numbers of nucleated reds and a leucocytosis will be found, and this followed by a jump in the red blood-count. The nucleated reds then disappear and the increase in the count is less rapid until perhaps another crisis occurs. V. Noorden reported cases with gains of half a million cells in four days. Since a leucocytosis is also present, he considered it a transitory increase of activity on the part of the bone-marrow to regenerate the blood. Normoblasts are, however, not the only red cells increased, and a crisis is not always a sign of improvement (see page 596). It is to be distinctly emphasized that the number of nucleated reds in the circulating blood is a poor index of the activity of the bone-marrow, much less so is the actual count of red cells. The bone-marrow may be able to maintain the count at the normal level through the most strenuous efforts.

INTERMEDIATE RED BLOOD-CELLS (Plate I., 31).—This is an exceedingly indefinite term; by it are meant cells which are not quite large enough to be called megaloblasts, and yet are larger than normoblasts; large cells with the nucleus of an immature red, or smaller cells of normoblastic size with a relatively large reticular nucleus. If one systematically measures all nucleated reds in a specimen, the small number of these cells is striking unless one include the immature nucleated reds of Howell (Fig. 113, *b*).

MEGALOBLASTS.—In the marrow always are found nucleated reds (Fig. 113, *f*), which are from two to four times the size of an ordinary red blood-cell. They are round or oval. The protoplasm makes up most of the cell, and is often polychromatophilic, but the polychromatophilia may be explained rather by youth than by degeneration;

in the case of the primary anæmias they are rich in hæmoglobin; in the normal marrow they are often pale. In stained specimens the nucleus is large, plump, round or oval, especially the former, usually central; in fresh smears it is easily seen, and has a good chromatin net-work if a good nuclear stain be used. It is thus seen that these cells are larger as a whole, and have a larger nucleus than have other nucleated reds, but what should they be called?

In all reports of blood cases it is necessary to know the writer's definition of a megaloblast, for opinions vary much. Some demand a large cell, some a large nucleus. Our rule is that both of these specifications must be fulfilled, and for reasons to be given later we ask that the size of the nucleus shall be at least that of an ordinary non-nucleated red (7.5 microns). Pappenheim and others consider that megaloblasts have no direct relation to normoblasts, and that from certain fine points concerning their nuclei a megaloblast may be recognized, even though it be as small as a normoblast.

The megaloblast of pernicious anæmia differs somewhat from the large nucleated red of bone-marrow. The size of both is about the same, but the normal bone-marrow megaloblast has usually a round nucleus with very distinct margin and chromatin net-work, while the nucleus of the megaloblast in the blood in pernicious anæmia is more often oval, much less distinct in fresh blood, stains much fainter in the smear, has less definite nuclear membrane and chromatin structure, and looks flabbier. But these differences are slight and inconstant.

The significance of the appearance of megaloblasts in the blood has been the subject of dispute; Ehrlich, considering that they never occur in the normal bone-marrow of an adult, believes them to be the product of a megaloblastic degeneration of this tissue due to a toxine, a reversion to the embryonic condition, and others say "to the amphibian," and that any attempts to break down the distinction between normoblasts and megaloblasts fail from the fact that in pernicious anæmia the blood is megaloblastic. (We are inclined to think that the expression "reversion to the amphibian type of blood" is much too often used. The only amphibian we have studied whose blood resembles that of pernicious anæmia is the batrachoseps, and one would hardly call an hæmoglobinæmia a "reversion," although that is the blood condition in some worms, hence could as easily be called a "harking back.") Such megaloblastic degeneration may explain their large numbers in the marrow of pernicious anæmia and some other conditions, but we fail to find any recent observer who has not easily found them in all normal marrows. In our study of bone-marrow, in which many nucleated reds have been measured, we have found the predominant cell the immature normoblast, with a nucleus between 3 and 4 microns in diameter. The next most common cell is a megaloblast, with a nucleus of 7 microns or over in diameter, or in one axis if oval. Between these large cells and the immature normoblasts occur every intermediate size, and yet the group of all of these is not as great as that of the large cells which constitute about 15 per cent. of the total number of nucleated reds. This applies to specimens with nucleated reds in moderate numbers, not those with innumerable nucleated reds in nests.

It has been shown that in certain animals the nucleated reds are grouped in islands, with the centre of megaloblasts surrounded by zones of cells of diminishing size to the periphery, where normoblasts are found (Bunting). The develop-



ment is not from centre to periphery alone, for in each zone, and especially the peripheral, signs of active regeneration are found, and each cell can produce its like as well as a smaller form. Ordinarily it is the periphery of these islands alone which furnishes new cells to the circulation, but if because of an over-demand the islands be encroached upon, the larger cells of the interior will be thrown into the circulation. Bunting has shown in a most interesting manner that by various blood poisons the peripheral zone of these islands may practically be stripped off. Should this occur in pernicious anæmia it is easy to understand the large numbers of megaloblasts in the blood, yet why nucleated cells reach the circulation in some cases and not in others is not clear, since in some cases the marrow is very rich in megaloblasts and none in the peripheral blood, and again the blood will show great numbers (blood crises). The bothriocephalus anæmia shows that certain specific toxins can produce this "megaloblastic degeneration" of the bone-marrow, for as soon as the worm has died the blood at once regenerates.

Karyokinesis of these large cells occurs in the peripheral blood, especially in severe anæmia, and is usually one of the terminal phenomena.

Thayer has seen definite amœboid movements in a megaloblast.

**MICROBLASTS.**—By microblast is meant a very small nucleated red, under 6 microns in diameter, with a small pycnotic nucleus. These occur in the circulation in severe traumatic anæmias, never normally; some appear perfect cells, and others as if pinched off from larger cells. The former may be the forerunners of microcytes.

The *fate of the nucleus* of the red has been the subject of great discussion, and still is. Two views have been held: (1) that the mature normoblast extrudes its nucleus (Rindfleisch, Howell, *e.g.*), and (2) the intracellular destruction by karyorrhexis and karyolysis (Kolliker, Neumann, *e.g.*). Those who hold this latter opinion admit that the nucleated red may even be seen to extrude its nucleus, but consider that this is pathological; that normally the nucleus goes to pieces in the cell, either by solution or by fragmentation, and the process which predominates varies with the disease. Many writers believe that all of these methods may obtain; Ehrlich, for instance, that the normoblastic nucleus is extruded, and that the macroblastic is absorbed; Pappenheim, that for both it is intracellular; Bloch, that either is possible. Whether or not the nucleus is extruded or disappears within the cell, the cell then flattens somewhat, becoming more disk-shaped and then biconcave. But not all are biconcave, some are spherical, especially in the embryo, a point emphasized by those holding the absorption view. The "degenerations" or nuclear fragments described by Vaughan (page 456) and by Cabot (page 510) indicate the intracellular type. Just at present the extrusion idea seems to obtain, but perhaps chiefly since so many need the nuclei to explain the origin of the platelets. The free nuclei which some emphasize in stained specimens may have been thrown out of the normoblast by the centrifugalized force of the sudden motion of the cells as the specimen is made, and the nucleus of the megaloblast remains in the cell, since its specific gravity is nearer that of protoplasm (Ehrlich, Pappenheim).

The *changes in the nucleus* are important. By a "pycnotic" nucleus is meant one diminished in size, dense, homogeneous, sharply defined, sometimes vacuolated, without any good chromatin net-work. The Ehrlich stained cells show no structure at all, only a deep uniform chromatin stain, but good nuclear dyes show traces. There seems a decrease of nuclear fluid and a solution of the chromatin in it. This is a preliminary step of karyolysis or absorption, the nucleus taking

a fainter stain till it cannot be distinguished from the surrounding protoplasm. It may precede karyorrhexis or fragmentation of the nucleus, which fragments may then disappear by karyolysis. The normoblastic nucleus may by amitosis divide into polymorphous forms, with two or even twelve fragments (Plate I, 34) of equal or unequal size, and usually united by a filament, giving beautiful rosette pictures. Some attribute this to karyorrhexis rather than to abortive mitosis. In a recent case during a blood crisis 55 per cent. of the erythroblasts were of this description, some having the nucleus in even twelve lobes. Another method suggested by some specimens is the disappearance of the most of the nucleus, leaving a few chromatin strands and masses. The study of nuclear degenerations should never be made with specimens stained with the Ehrlich stain. That is least adapted for nuclear changes. One other point well seen in the bone-marrow is the varying hæmoglobin tinge of the corpuscles, these cells showing a much wider variation than those of the blood.

This could be explained on the ground that the development of hæmoglobin was an intracellular matter, a gradual process. Some cells seem to reach complete hæmoglobin development after they lose their nucleus, some before. The question is of interest in the study of anæmias. Are these pale cells in the circulation of fixed composition, or only immature and later develop more hæmoglobin? Can a normal cell lose some hæmoglobin; that is, can the color-index rise and fall, and yet only the same cells remain? When the color-index falls is it because the cells are like depreciated currency, and it rises when these are recalled and better issued in their place? The question may have little practical importance, and yet if discussed the much desired result should be greater care in the use of terms with which we describe our cases. Arguments from comparative anatomy are not satisfactory, yet the evidence goes to show that in lower vertebrates the red cells can complete their development in the circulation, while in the mammals an imperfect cell is said to be incapable of further development. Among others, Gaule and his pupils believe in a hæmoglobin "store" in the body, which in case of need is carried into the circulation in new corpuscles and returned when the extra cells are withdrawn.

We fear the great trouble is in the hæmoglobinometers, and that until careful work is done with the best instruments the question will remain unsettled.

ORIGIN OF RED BLOOD-CELLS—That the ordinary non-nucleated red blood-cells come from the nucleated reds is now doubted by few. Up to the end of the fourth week of embryonic life all of the blood-cells are nucleated. From that time on the number of the non-nucleated cells increases until at the third month only about one-sixth to one-eighth are nucleated. At the fifth month they are still numerous, but at birth it is rare to find any nucleated red cells in the blood.

In the earliest embryonic life the vessels are formed from solid cords of cells, the peripheral ones of which become the endothelial lining of the vessel wall, the internal cells the corpuscles. This process may occur in almost any part of the developing organism, and perhaps also in the adult when there is new formation of blood-vessels. In addition, in the embryo many mitoses are found in the nucleated reds of the circulating blood.

Before the third month the liver has become the chief seat of blood formation; after the fifth month the spleen and the lymph-glands take up the task; and at last the marrow becomes the chief organ. In the child the marrow of the whole skeleton has this function, but at about puberty and during adult life only the ribs and some of the flat bones. Howell considers that callus, for instance that fol-

lowing a fracture, may in the adult also for a while furnish centres for hæmogenesis. In the adult it would seem as if the spleen could resume this function in leukæmia and anæmia.

Removal of the spleen causes little anæmia, but after about a month begins a long continued rise of small mononuclears, and in some conditions the blood is even leukæmic, and then after about twelve months an eosinophilia of even extreme degree. These phenomena are now explained as manifestations of the vicarious activity, first, of lymph-glands then of the bone-marrow, for the spleen. The chief function of the spleen seems to be to remove old reds and leucocytes from the circulation, and the acute spleen tumor in some conditions is due to the great number of leucocytes ingested (spodogenic splenic tumor).

In the embryo the blood-cells are at first without hæmoglobin. At this time there are no true leucocytes and none appear until after the formation of red cells is active. The embryologists have shown that before the appearance of leucocytes in disease of the embryo the red blood-cells are amœboid, perhaps phagocytic, which is interesting, since in certain blood diseases of the adult there is at least a suggestion of these two functions.

In lymphatic leukæmia (certain acute cases) the view is still held by some that the increased cells are "red cells" without hæmoglobin, and the converse Pappenheim holds to be true in severe anæmias, some of the large lymphocytes developing to megaloblasts instead of to small lymphocytes.

Howell was the first to demonstrate in the cat "ancestral corpuscles" which resemble the red blood-cells of reptiles, being large, oval, semifluid red cells, with a deeply stained oval nucleus. These cells were later described by Engel as "*metrocyles of the second generation*," those of the *first generation* having a large chromatin-rich nucleus. Later such cells never, normally at least, reach the circulation, and Engel thinks they are no longer formed.

For the study of the young red cells the blood of embryo mice is to be especially recommended. Here a great variety of changes in the nucleus and in granulation of the protoplasm may be demonstrated.

WHITE BLOOD-CELLS.—For the best recent study of bone-marrow, see Schur and Löwy.<sup>43</sup>

#### A. GRANULAR CELLS

##### I. Neutrophiles. Neutrophile myelocytes.

1. Typical myelocytes; cells from 12 to 15 microns in diameter, with a large round nucleus, the protoplasm scanty, forming often a thin rim around the nucleus, and finely granular. These cells are by far the most numerous in the marrow, but on account of their size seem even more so than is the case. The nucleus is often hard to make out. In the bone-marrow may also be seen beautiful larger myelocytes with faint nuclei, which, however, are not often found well preserved since they go to pieces so readily, and which occur in the blood only in leukæmia, "Cornil's marrow cell." In the fresh marrow some of the myelocytes have a very small, dense, round nucleus, which we think is a post-mortem change from the loss of nuclear fluid. All transitions from those cells with a round nucleus to the typical leucocytes are present on the one side, all transitions from large mononuclears with but few or no granules to those full of granules, on the other.

<sup>43</sup> Zeitschr. f. klin. Med., Bd. 40.

2. Cells similar to the above, but much smaller. The nucleus indented, or slightly polymorphonuclear, but staining faintly,—“transitional cells.”

3. Typical leucocytes.

II. Eosinophiles, always relatively few in number.

1. Eosinophile myelocytes. Large cells with pale nucleus, scanty protoplasm filled with eosinophile granules, otherwise similar to the above mentioned neutrophile cells.

2. Similar to the above, but smaller; the nucleus indented or slightly polymorphous. All gradations may now be found to typical

3. Eosinophile leucocytes.

III. Basophiles.

1. Mastzellen, which, however, are rather rare to find. These cells have nuclei of a variety of shapes, yet usually polymorphous (see page 536). Mononuclear Mastzellen occur (Engel). These are, at least, hard to recognize, since the young  $\alpha$  and  $\epsilon$  granules are quite basophilic.

2. Various cells with violet granules, which vary in size. The cells are rather small. These, although containing basophile granules, are not typical Mastzellen. In this connection it should be mentioned here that the  $\beta$  granules may occur in the eosinophile cells or by themselves.

3. Polymorphous cells, with fine basophile granules. These may be neutrophile cells stained by the “tricky” methylene blue (Schur and Löwy).

#### B. NON-GRANULAR CELLS

1. Lymphocytes. Size of red blood-cells, nucleus rich in chromatin, protoplasm a narrow rim. These cells are the second most numerous cells and often seem naked nuclei.

Among these are the “protolymphocytes” of Osler, solid-looking lymphoid elements from 2.5 to 5 microns in diameter, which resemble free nuclei; some have a rim of protoplasm. From these “erythroblasts” develop (see page 549).

2. Medium-sized lymphocytes with more protoplasm and a smaller often eccentric nucleus.

3. Very large cells with general character of lymphocytes which occur in the blood in some acute leukæmias, but none normally; “Large lymphocyte” (Ehrlich, Fränkel, Pappenheim); Grawitz’s “unripe cell.” Wolff’s “indifferent lymphoid cell,” Naegeli’s “myeloblast,” Troje’s “marrow-cell.” The nucleus stains faintly, is seldom lobulated, is very pale and poor in chromatin, the protoplasm is faintly basophilic.

Also to be mentioned are cells, common enough, which in the fresh exactly resemble normoblasts (immature), except they have no

hæmoglobin. The nucleus is that of Howell's immature red, perfectly round, with sharp margin, distinct chromatin net-work, and clear hyaline protoplasm. They are from 9 to 12 microns in diameter. These are the erythroblasts of Osler, Löwit, and Howell.

Löwit described as "leucoblasts" cells with relatively large nuclei containing one or two chromatin masses which are sometimes irregular in shape, and from which a system of delicate lines and bands radiate to the nuclear membrane, which membrane is distinctly doubly contoured, and has on its inner surface projections which are connected with the infranuclear net-work. Concerning their method of division, upon which Löwit lays much stress, it is not easy to decide.

Ehrlich believed the true lymphocyte came from the lymph-glands; others say from the bone-marrow. Some have tried to distinguish morphologically those from the marrow from those from glands (Rubenstein). The question now is, "Do any come from lymph-glands?" thus admitting that typical "lymphocytes" are an important constituent cell of the marrow. Michaelis and Wolff tried to differentiate these cells on the basis of their future history, the lymphocytes from lymph glands remaining such, the "lymphoid" cells of the marrow being capable of further development to a granular cell. But this capability does not aid us much in saying which the individual cell now before us is, although these writers did describe slight differences in their staining reactions. And yet this distinction between lymphocytes and lymphoid cells is probably just. Many workers fall back upon such indifferent lymphoid cells as young forms, naming them "protocleucocytes" (Osler), etc., and consider that from them develop colorless cells which correspond to the leucoblast and erythroblast of Löwit and Howell, and from these the whole series of reds and whites. The lymphocyte with diffusely staining notched nucleus (Rieder's cell) is probably an old form of lymphocyte. The small mononuclears with round vesicular nucleus, delicate chromatin net-work, and rather broad band of basophilic protoplasm with smooth margin, are young cells which resemble only in appearance those of the normal blood, and themselves do not belong there. The lymphocytosis of young babies is unquestioned, and yet the most rational explanation for this is that there is an overproduction of leucocytes which did not exist at birth, since then there was no function for the cell. Yet this would mean that young leucocytes are lymphoid cells. The same might be said for the lymphocytes of the digestive leucocytosis.

Among marrow cells are forms which never reach the circulation; probably young (also old?) leucocytes and red blood-cells, and perhaps undifferentiated cells the ancestors of both series, unless one agrees with Bizzozero that none of the ancestors of red cells are without hæmoglobin. The variety of forms of cells found is so great that not one sharp dividing line can be drawn to separate a single group, and one may find evidence in favor of any ancestral tree he wishes. Two main views are held, the one that the youngest cells in the series are very large, with large, faintly staining nuclei, and protoplasm which very quickly goes to pieces, hence they are not always found; the other that they are small. According to the former, in each succeeding generation the cells become smaller and more resistant; the large fragile mononuclears develop granules and become large myelocytes, in the next generation are ordinary myelocytes, and these give rise to leucocytes. Of course, too much stress may have been laid on the shape of the nucleus as an age sign; it may be that a certain amount of irregularity in the nucleus is an expression of an amoebic cell, and that the mononuclear granular cells in some exudates are leucocytes which have resumed a resting form. The same large white cells may develop hæmoglobin in their protoplasm and become megaloblasts, the succeeding generations of which are the intermediate, then immature nucleated reds, then the mature, then the ordinary red corpuscles.

The other view considers small lymphoid elements, some even like naked nuclei, as the youngest cells, from which, by increased size and differentiation, myelocytes and erythroblasts arise, then to again diminish in size with advancing age.

Most will now agree that there is a large group of indifferent cells which will develop in whatever direction (red or white) necessity demands. The only question is, Which are these cells?

The development is more by "steps" than by a gradual transition, and those of each step are able to produce others of their kind as well as those of the succeeding generation (in point of size, etc.). The picture is still more complicated, since the line of descent of these cells is not single, but new ancestors can be found at each step in the progress, so that to trace backward is more like following a stream toward its source. It is a single river at its mouth, but as we go toward its source many tributaries are found which contribute to its volume. Thus Pappenheim traces normoblasts from small lymphocytes, megaloblasts from large lymphocytes, and considers the polychromatophilic group evidence of the transformation from a basophilic lymphocyte to a red cell; subsequent workers trace normoblasts from megaloblasts. Normoblasts certainly can produce normoblasts, and megaloblasts megaloblasts. Again the granulation may appear in cells with nuclei at various stages of deformity, as if the changes in the nucleus from round to polymorphous bore little parallelism to the development of granules.

We cannot here take up the question of the origin of leucocytes and perhaps red cells in other organs. The above remarks are not intended as a *résumé* of the subject, but an answer to many of the questions suggested to students by the study of bone-marrow. We merely mention Nothnagel's case of general osteosclerosis, with the entire marrow practically functionless, yet a normal count of neutrophiles; also the presence of mononuclear granular cells in areas of inflammatory infiltration.

4. Pigmented cells, often absent.

5. Giant-cells.

(a) Megalokaryocytes with one large irregular coiled nucleus.  
"Giant-cells with budding nuclei."

These are the "hæmatoblasts" of Foa and Salvioli, which they say give rise to smaller hyaline cells, which develop hæmoglobin to form nucleated reds. These cells are seldom found in the stained specimen, although masses of detritus which one may suspect to arise from them are found. These cells occur in the circulation in a leucocytosis, and are filtered out in the lung (see Plate II, 11).

(b) Multinuclear cells. Osteoclasts.

Many cells are seen, especially in fresh specimens, with very interesting degenerations and inclusions. Some large mononuclear cells contain large globules or droplets resembling myelin, droplets about 3 microns in diameter, rather uniform in size, and with the yellowish shimmer of the myelin droplets of the sputum cells. Some cells are filled with very large granules with the color and refractivity of a granule (see Fig. 115, e). Such cells Howell found in the marrow of the cat in good numbers, and considers them to play an important part in metabolic changes in the marrow. In other cells occur globules of fluid, giving them a vacuolated appearance. Large "dropsical" projections both of protoplasm and of nucleus are also seen.

Unlike the red cells, in the leucocytes it is much easier to trace the degenerations. In normal blood practically all the leucocytes are normal, but when there is a leucocytosis or especially in the leukæmias many cells are seen concerning the death marks of which there is little doubt. The lymphocytes are almost devoid of protoplasm, the nucleus small pycnotic and indented, or even polymorphous (Rieder's cells) (Plate II, 17). The polymorphonuclear granular cells have nuclei very pycnotic and fragmented, although probably a chromatin thread always connects the fragments. For an interesting manner in which the neutrophile leucocytes may be classified, based on the number of nuclear fragments, and the use to which such a classification may be put, see the publications of Arneith.\*

Concerning the large pale nuclei without protoplasm there is doubt, since they could be the very sensitive young cells destroyed in the preparation of the specimen. But the similar changes in the small mononuclears, which may in leukæmia be a feature of even the majority of the cells, suggest very strongly that they are degenerations.

Late in leukæmia it would seem (said Ehrlich) as if the ability to develop neutrophile granules might be lost, and clear cells are found which resemble the granular cells in every way except they have no granules.

All these questions would be much less interesting if the cells have the ephemeral history which some ascribe to them. Winternitz (quoted from Grawitz) estimated that in the dog the lymph supplied the blood through the lymph-duct daily a number of lymphocytes equal to more than half the total number in the body. If this be true, the chief function of most of the cells must be to increase the proteid content of the plasma. A similar question is the source of the pus-cells in cases with great pus formation, as cystitis and bronchitis or bronchiectasis, in which by actual estimation the person loses daily a number of white cells almost equal to the total number in his circulation at any one time.

**Fœtal Blood.**—In the three-months' human embryo Engel found nucleated red cells of normal and large size, "metrocytes of II. Generation"; that is, large spherical nucleated reds, 12 to 20 microns in diameter, rich in protoplasm, the nucleus relatively small, 3.5 to 6 microns in diameter; but in some cells 17 to 20 microns in size the nucleus was 7 to 8 microns. These cells occurred in frequency of from 4 to 6 per 100 normal reds. (Metrocytes of I. Generation he describes from mouse embryo's blood as spherical cells from two to three times the size of a normal red cell, the nucleus often in mitosis and filling but a relatively small part of the cell; this, he says, is not a megaloblast nor a gigantoblast. At this stage there are no non-nucleated reds and no leucocytes.) At this stage occur two forms of normoblasts,—those staining orange, from 5 to 9 microns in diameter and the nucleus 3.5 to 5 microns; those staining red (Ehrlich stain), about 7 to 8 microns in diameter, with a relatively large nucleus rich in structure 5 to 6 microns large, the protoplasm scanty and ragged; in this latter group are some large cells 16 microns in diameter and a nucleus of 11 microns; these are Ehrlich's megaloblasts.

The other cells were free metrocyte nuclei, lymphocytes, neutrophile myelocytes, and leucocytes.

In embryos of 6 cm. length the non-nucleated reds were to the nucleated as 12:1; of 12 cm. embryo, 55:1; of 16 cm. 150:1; of 19 cm. 176:1. In the 6 cm. embryo the metrocytes were 4 per cent. of the reds; in the 12 cm., 0.25 per cent., and later none. The leucocytes in the 6 cm. embryo were to the reds as 1:500 to 1000.

Engel admits that embryos of the same age differ so that he could not tell the age of the embryo by studying its blood.

We have had opportunity to study the blood of a fœtus 15 cm. long, and found red cells, 1,168,000; hæmoglobin, 25 per cent.; leucocytes, 9000. Nucleated reds, 1:19 of total reds, normoblasts and intermediates, beautiful polychromatophilia.

In an embryo 20 cm. long we found reds, 2,652,000; leucocytes, 28,000; hæmoglobin, 38 per cent.

In an embryo of 23 cm. Engel found the reds (heart's blood), 3,300,000; hæmoglobin, 80 per cent.; leucocytes, 40,000. Nucleated reds were to non-nucleated as 1:120, and all normoblasts. Of the leucocytes, the granular were to the non-granular as 2:5; neutrophile myelocytes and leucocytes present with all transitions, and a few eosinophiles.

The blood of a 27 cm. embryo contained nucleated and non-nucleated reds in relation of 1:200, leucocytes to erythrocytes as 1:90, polymorphonuclears to mononuclears as 4:5.

**Leucocytosis.**—By this term was meant an increase above normal of the white cells of the blood, but the term now means a transi-



tory, symptomatic, absolute increase of the polymorphonuclear neutrophiles especially, in the peripheral blood, above the maximum that is normal for a given individual in the condition in which he at that time finds himself.

In general 10,000 leucocytes per cubic millimetre is the limit an increase above which is said to be pathological. But the matter is a relative one, and only when so considered does the condition have the clinical value claimed for it. Some persons have normally a leucocyte count of 10,000 to 12,000. The count also depends on the condition of the person. For instance, if cachectic with a leucocyte count of 4000, a rise to 8000 would mean as much as a rise to 20,000 would for some normal persons. This was beautifully exemplified in one case of typhoid fever with a leucocyte count of 1600. A parotitis developed and the leucocytes promptly rose to 3200, a true leucocytosis for that person at that time.

A leucocytosis also is transitory and symptomatic, and this distinguishes it from leukæmia.

The term leucocytosis now has a very special meaning. It is used of an absolute increase of the polymorphonuclear neutrophile cells. An increase of one of the other types of white cells is named according to the cell increased; for instance, if it is the mononuclear non-granular cells, lymphocytosis; if the polymorphonuclear eosinophiles, eosinophilia; if the mononuclear granular cells, myelæmia, etc. It is very seldom that one group of cells alone is increased; usually others are to a less degree; but since there is evidence that the various cells are not all related very closely at least to one another, it is their absolute number which is to be considered rather than their relative, that is, than their percentages or "formula." With even a diminished per cent., providing the total count be raised, the absolute number may have increased, while the reverse also is true that when the percentage seems to indicate an increase the absolute number may have dropped if there is a diminution of the total number.

Or a group of cells may remain unchanged, while other groups change much. A good illustration of this is the following, a case of Frazier and Halloway: Count, 13,040; polymorphonuclears, 78.2 per cent. (*i.e.*, 10,197); small mononuclears, 16.8 per cent. (2191). The total count rose to 54,960; polymorphonuclears, 90.4 per cent. (49,684); small mononuclears, 4 per cent. (2198).

The "general type" of leucocytosis—*i.e.*, an equal increase of all the leucocytes—is rarely seen. It results from stasis of blood in the capillaries, following a cold bath, or massage,—*e.g.* Also cases of the digestive leucocytosis and that of pregnancy, *et al.*, show it in some degree.

Classification (Limbeck).—1. Physiological: (a) Digestion; (b) Pregnancy; (c) Newborn. 2. Pathological: (a) Inflamma-



tory; (b) Malignant tumors; (c) Post-hemorrhagic; (d) Agonal.  
3. After medicinal and therapeutic measures. 4. Various other causes, as shock, etc.

**Digestion Leucocytosis.**—The leucocytes of a normal person who after a fast of twelve or more hours partakes of a rich proteid meal will usually rise to about one-third above the normal number. The count begins to rise in about one hour as a rule, reaches a maximum in from three to five hours, and then decreases. While the polymorphonuclear neutrophiles are especially involved, the small mononuclears are to some extent, in some cases considerably. For some persons no preliminary fast is necessary; in others the leucocytes do not rise at all. (Limbeck thinks habitual constipation explains the latter.) Children show it more markedly than adults, and the well nourished than the poorly nourished; it is greatest in the infant after his first meal of cow's milk. For the nursing infant it is said to be absent, and hence the opinion (Moro) that it is a reaction against foreign proteid. A rich proteid meal is necessary, hence diabetics show it well. Particular stress should be laid upon this point, that the meal should be unusually large, for the leucocytes are even fewer after a light meal (also after some heavy ones), or again they may not change. It is absent in the herbivorous animals, and little in man after a vegetable meal.

The explanation is in doubt. One thing is quite certain, that it is due to the absorbed products of proteid digestion, which have a positive chemotactic influence. Hofmeister suggests the proliferation of the large masses of lymphoid tissue along the intestine, due to the stimulation of the digestive processes, to be the cause; hence it is a mixed leucocytosis. The lymphocytosis is, however, not always present.

Jaffé says that in children the leucocytosis is not dependent on the meal, but is periodic.

The reverse relation is also true. Persons in starvation show a low leucocyte count; Succi, who fasted seven days, had a count of 861 per cubic millimetre, while the insane with melancholia often have counts below 3000. On the other hand, well-nourished persons often have counts from 10,000 to 12,000.

The function of the leucocytes is probably not alone protective, but they play an important part in absorption, transportation, and assimilation of food; hence their number depends much on the age and nutritional condition of the person.

There is some value in the digestion leucocytosis to aid in differentiating between pernicious anæmia and cancer of the stomach. In severe blood diseases, pernicious anæmia, and in ulcer and other gastric diseases it is present, while in cancer of the stomach even fairly early, it is sometimes absent, but not always. It is absent in

some benign conditions.<sup>45</sup> Gastric catarrh and involvement of the lymph-glands are given as its explanation. We wish to emphasize the necessity of giving a rich proteid meal and of counting the leucocytes once an hour. Only a considerable rise is of value.

**Leucocytosis of Pregnancy.**—About 75 per cent. of women during the last months of pregnancy show a count above normal, an average about 13,000 per cubic millimetre. This is especially true of primiparæ, and yet the explanation is more the youth and the nutritional condition of the patient than the fact that she has had no previous pregnancies. The count rises until the end of pregnancy, and then diminishes in from four to fourteen days after delivery. The differential count may remain practically normal, yet it is the polymorphonuclear neutrophiles that are especially involved. In multiparæ there is also a rise, but it is within physiological limits.

The explanation has been disputed. It is agreed that it is not the pregnancy *per se*. V Limbeck considers it a prolonged digestion leucocytosis, due to the need of additional nourishment for the mother and child. In favor of this is the absence of a digestion leucocytosis or even a diminution of the count after a heavy meal, due, it is said, to a migration of leucocytes to the placenta, where is the greatest accumulation of the positively chemotactic products of digestion. The condition of the breasts is also suspected; others ascribe it to an overactivity of the lymphatic system. But the view most commonly held now is a slight autointoxication, against which the primipara reacts better than a multipara. Thomson found that of 33 counts on twelve pregnant women made during the eight months of pregnancy but one was below 7000; the highest 13,200.

But the question is, What is the usual count for a normal woman? Is it 5500? If so, pregnancy causes in all cases a relative rise, and in most an absolute leucocytosis.

Zangemeister and Wagner<sup>46</sup> think the question not quite so clear. Of 47 normal non-pregnant women, all under practically the same conditions, from twenty-one to thirty-four years of age, 35 (74 per cent.) had a count above 10,000 (mean about 12,500). The leucocytes of pregnant women (57 cases) varied within the same limits as non-pregnant (70 per cent. above 10,000; mean count between 12,500 and 15,000), nor did the number of previous pregnancies seem to make any difference. The counts which these writers report are about the same as those of the writers claiming a leucocytosis as a feature of pregnancy, only the former claim that normal non-pregnant women give the same. During labor, of 63 cases there was a rise even to three times the previous count in nearly all cases, with the maximum at or just after delivery. This was especially marked in cases of prolonged labor or of those who suffered greatly. In quick, easy labors the rise is insignificant.

In 75 cases during the puerperium there was a rapid decrease to normal. On the seventh or eighth day an increase of mononuclears, with the involution of the uterus (Rouslacroix and Benoit). The study of two cases of version led them to think the cause of the rise was the contractions of the uterus.

In pathological cases the leucocytes give no aid in diagnosis or prognosis, since as high counts are seen in the physiological cases.

Lobenstein<sup>47</sup> considers that there is a leucocytosis of pregnancy, the average of 50 cases during the ninth month being 11,854 for primiparæ, and 9346 for multiparæ; and on the third day of the puerperium, 13,200 for primiparæ, and 11,600

<sup>45</sup> Rencki, Arch. f. Verdauungskr., Bd. vii.

<sup>46</sup> Deut. med. Wochenschr., July 31, 1902.

<sup>47</sup> Am. Jour. Med. Sci., 1904, vol. cxxviii. p. 281.

for multiparæ. These figures are too nearly normal to name them leucocytoses. In 20 cases the digestion leucocytosis was tried, found present in 13, but an actual diminution in the count in 6. Of 13 cases of eclampsia, in 6 mild cases the highest count was 31,000; in 6 severe, 40,000 to 50,000; and in one severe case, 106,000 and death. He concludes that the leucocytosis is roughly parallel to the degree of intoxication and to the resistance. A low count and a rapidly falling count are bad signs.

**Leucocytosis of the Newborn.**—Although the foetus has so many blood-building organs yet the leucocyte count is very low, since as yet there is no function for these cells (Askanazy). The statement usually made is that at birth there is a leucocytosis of from 17,000 to 21,000, and after the first feeding a rise to from 26,000 to 36,000, with the increase chiefly in the number of small mononuclears. Examination of the infant's blood exactly at birth, however, in case the teacher wishes to demonstrate a true lymphocytosis, will assure the disappointed one that this is by no means the case, and he will usually find a condition of the leucocytes quite like that of the adult. The question has been studied by Gundobin, Carstanjen, and Warfield,<sup>48</sup> with the following results. On the first day after birth the average leucocytosis is about 26,000 (11,700 to 34,700); on the third day, the average is 13,270, and on the eleventh day 15,740. For the first few days there is an absolute increase in the number of polymorphonuclear neutrophiles, with a percentage of 70.42 on the first day, 53.16 on the third, and 34.2 on the eleventh. The large mononuclears and transitionals are high, being 10.76 per cent., 16.67 per cent., and 15.98 per cent. respectively on these three days. The eosinophiles vary much; Mastzellen and myelocytes are few and rare. It is not until the eleventh day that the count which is usually considered normal for infants, with 40 per cent. small mononuclears, appears.

This high count of the leucocytes has been explained by a concentration of the blood or a digestion leucocytosis, but the more rational explanation is the rapid blood formation at that age. Although normal infants vary much, yet this rather high count may continue until from the third to the sixth year, after which time the blood picture of the adult prevails. During these early years the polymorphonuclear neutrophiles vary from 18 to 40 per cent., the small mononuclears from 40 to 60 per cent. of the total number, and often there is a slight increase in the eosinophile cells.

**Leucocytosis of Inflammations and Various Febrile Diseases.**—There is an absolute increase of the polymorphonuclear neutrophile cells especially accompanying most inflammations, most acute infections, and other febrile diseases, which is roughly parallel to the temperature, and which depends especially upon the activity of the inflammatory process and the condition of the patient.

<sup>48</sup> Amer. Medicine, September 20, 1902.

The following general statements may be made. Whatever the immediate cause, a leucocytosis represents the reaction of the individual to the disease. In those conditions usually accompanied by a leucocytosis a high count means a vigorous reaction, little more; a low count may mean a poor reaction, hence indicate a poor prognosis, or the infection may be of so mild a degree that it can elicit little or no reaction.

On the other hand, diseases differ in their ability to produce a leucocytosis: some do practically always, as pneumonia, and in a degree roughly parallel to the virulence; some never, as measles, malaria, and tuberculosis; some perhaps an early leucocytosis followed by a leucopenia, claimed for typhoid fever, but doubted by most; some none at first, then a rising count, as typhus fever, some cases of influenza, and smallpox. Some diseases ordinarily without leucocytosis may in cases of great severity show one, as malaria. In certain cases much depends on the situation of the infection, as in typhoid fever, which infection, when it causes empyema or periostitis, is accompanied by a rise of leucocytes; also tuberculosis of the meninges, and caseous pneumonia.

In cases of local infections, as abscess formation, the leucocytosis is a symptom related to the fever and other toxic features, and evidently like them caused by, and its severity determined by, the toxine absorbed; for following operation and free drainage both quickly drop to normal. For much the same reason the count runs quite parallel to the richness of the exudate in pus-cells.

It is not the exudate formation alone which governs the leucocyte count, for cases with free drainage of pus may lose enormous numbers of white cells daily (almost as many as are in the circulation at any one time), and yet show a normal count. This is well seen in some cases of chronic bronchitis, bronchiectasis, cystitis, etc., in various bone and joint abscesses with discharging sinuses, in empyema after operation, etc. The agent causing the leucocytosis seems the same as that causing fever, for they usually begin and end together, depending on the free drainage of the exudate.

Of course one would expect that a great loss of cells in an exudate would mean a diminution in those of the blood, and in acute cases, a spreading peritonitis for instance, this is thought to be the explanation of the sudden drop in the count of the blood.

In general, the leucocyte count runs in no way parallel to the severity of the condition; a simple local felon may cause as high a leucocytosis as an appendix abscess, and a fatal pneumonia as little rise as a boil.

Among the conditions causing leucocytosis are: *Acute lobar pneumonia*, the best studied (page 629).

*Acute tubercular pneumonia* (page 624).

*Acute articular rheumatism* (page 633).

*Diphtheria* (page 623).

ACUTE CEREBRO-SPINAL MENINGITIS caused a leucocytosis in all of 21 cases (Osler); in 4, over 40,000; the highest, 47,000. The leucocyte count is of no especial value in distinguishing the various forms of meningitis, since it is also present in the tuberculous.

An ordinary ACUTE FOLLICULAR TONSILLITIS usually causes a leucocytosis. This was true of 18 of 26 of our recent cases (12, from 10,000 to 15,000; 3, above 20,000; the highest, 27,000). There was considerable fever in all the cases with high counts.

*Scarlet fever* (page 623).

*Mumps*. The occurrence of a leucocytosis is disputed.

In WHOOPING-COUGH the leucocytes, especially the lymphocytes,

are increased three or four times the normal amount, averaging 40,000, the degree of leucocytosis depending on the severity of the case and its complications. It is more pronounced the younger the child is. The early appearance of the leucocytosis is important in diagnosis. The rise is chiefly of the lymphocytes, but not entirely. It begins with the disease, during the catarrhal stage, and continues longer than the paroxysms, is maximal during convalescence. Others claim it is a true leucocytosis; again others, that the formula is little disturbed.

RABIES sometimes causes a true leucocytosis of even 25,000, with 98 per cent. pmn. n.

ERYSIPELAS causes a leucocytosis which runs fairly parallel to the temperature, of 10,000 to 20,000 in mild cases, 20,000 to 30,000 in more severe. Its polymorphonuclear neutrophile character is more marked in adults than in children. These cells may be 92 per cent. in fatal cases. As the count falls, the eosinophiles may rise considerably.

In 6 cases the leucocytes were normal in 2, moderately elevated in 2, and 26,000 and 34,500 in the other two. The red cells were normal in all.

In ACUTE ULCERATIVE ENDOCARDITIS the leucocytes are high as a rule, especially in those cases running a protracted course, an important point in diagnosis, any long continued leucocytosis suggesting this. In rapidly fatal cases there may be no rise.

In 6 cases recently at death the count stood 7070, 13,600 (it had fallen from 34,000), 17,000, 47,000, and 48,000 (it had risen from 9800). In another case, 12,000.

In INTESTINAL OBSTRUCTION the leucocytes rise rapidly to about 16,000 when partial, to 20,000 or more when complete; with over 20,000 cells within the first twenty-four hours the chances are in favor of gangrene. This rise of leucocytes may be of value in a case of suspected post-operative obstruction. (Bloodgood.)

Following a thyroidectomy the MYXŒDEMA is accompanied by a count of even 49,000.

*Smallpox* (page 623).

CHOLERA.—At the algid stage the leucocytes may number from 40,000 to 60,000, and rapidly disappear during the stage of reaction.

PYOGENIC INFLAMMATIONS of the serous membranes, meninges, pleura, pericardium, peritoneum, not tuberculous, are accompanied by a leucocytosis which bears some relation to the cellular richness of the exudate in leucocytes, more to the fever. The count varies with the progress of the disease, since it may drop to normal while the process is stationary even if the temperature remains elevated, until a slight

spreading of the process causes a rapidly rising count. This is well seen in pelvic inflammations.

In 99 cases of PLEURISY WITH EFFUSION the red cells were practically normal; in 65 the leucocytes were below 10,000 cells, and in but three of the remaining were they over 15,000. Cabot reports almost exactly the same figures for the Massachusetts General Hospital (314 cases; 33 per cent. above 10,000; 6 per cent. above 15,000). The low counts are interesting since so many such cases are clearly tuberculous.

In EMPYEMA, on the other hand, there is almost always a leucocytosis, except in cases (14 per cent.) allowed to remain without operation for some time.

In 37 cases of ACUTE FIBRINOUS PLEURISY the leucocytes varied from 10,000 to 22,900 in 24. In the rest the count was normal.

INFLUENZA is a term applied to a wide group of cases, but with the diagnosis seldom confirmed by cultivating the organism. The demonstration that many cases of bronchiectasis and chronic bronchitis are really la grippe throws considerable doubt on the figures given of the blood-findings. But accepting the diagnoses as they stand, the leucocytes are normal in about two-thirds of the cases (Cabot), moderately increased in the rest. Blum<sup>49</sup> states that in the typhoidal or abdominal form there is leucopenia. Gerber<sup>50</sup> states that the leucocytes rise not at the height of the disease, but as the fever falls; that a count of 20,000 cells indicates pneumonia. During the rise the eosinophiles decrease or disappear.

In almost half of our cases the count was above 10,000 at the height of the disease, reaching even 25,000. What is of more interest is that nearly all the cases in which several counts were made showed early a very low count, then a sharp rise, which fell after the temperature was normal. This may explain why in the cases with but one count the leucocytes may be low, even 3000 to 5000 when the temperature is 100° to 105°, and high when the temperature is normal. It also shows that for diagnosis it is not one count that is of value, but the leucocyte curve.

Any *pyogenic processes of mucous membranes* accompanied by fever may cause a leucocytosis, as enteritis, urethritis, etc.

ACUTE BRONCHITIS is accompanied by a leucocytosis which continues as long as the fever. The count was from 10,000 to 20,000 in 30 of our 67 cases.

In CHRONIC BRONCHITIS the emphysema and attending cyanosis may explain the few cases with slight leucocytosis, present in just half of our cases.

The red counts averaged high, the mean being 5,000,000. Of 25 cases, 3 were above 7,000,000 (maximum 7,900,000).

In a case of true FŒTID BRONCHITIS, 22,500.

In 11 cases of BRONCHIECTASIS the leucocytes were 20,000 in 2;

<sup>49</sup> Wien. klin. Wochenschr., 1899.

<sup>50</sup> Wien. klin. Wochenschr., 1900.

between 10,000 and 20,000 in 4; normal in 5 afebrile cases.

Among the local pus processes in which the leucocyte count is an advantage are *appendicitis* (page 633), *pelvic inflammatory disease* (except tuberculous, and mild in gonorrhœal), *abscess of the liver*, *empyema of the gall-bladder*, *ovarian abscess*, *abscess of the brain*, etc.

In ABSCESS OF THE LUNG counts as high as 60,000 have been reported. In our 3 cases they were 8,100, 12,300 and 12,500. In two cases of GANGRENE OF THE LUNG they numbered 20,000 and 48,000.

In 25 cases of GONORRHOËAL ARTHRITIS, the mean red count was 4,500,000; the lowest was 3,600,000. The mean leucocyte count was 9,000. In 8 of 23 cases the count was between 10,000 and 20,000.

In PERIRENAL ABSCESS, 5 cases, the leucocytes varied from 19,000 to 36,000; PYELITIS, 4 cases, from 10,600 to 19,500. In PYELONEPHROSIS, 2 cases, 18,000 and 28,500; HYDRONEPHROSIS, 2 cases, 6,400 and 9,000; PYELONEPHRITIS, 1 case, 8,000. In RENAL CALCULUS, 4 cases, the leucocytes during the colic were from 12,000 to 18,000.

In GOUT the red cells are practically normal, 5,000,000 or over in all but 2 cases (of 13 cases the lowest was 4,300,000). The leucocytes rise with the onset of an acute joint attack. (In 18 cases there was a mild leucocytosis,—10,000 to 14,000 in 7 cases.) The variations in the leucocyte count run parallel to the febrile and joint symptoms.

There is a leucocytosis in DIABETIC COMA and in URÆMIA.

In DEMENTIA PRÆCOX \* there is a polymorphonuclear neutrophile leucocytosis which is coincident with the onset of abnormal phases, and for which no satisfactory explanation can as yet be given. It is not an accompaniment of fever and is not due to physical struggling. No counts higher than 15,000 have been reported. In GENERAL PARESIS there is often an absolute lymphocytosis. The total number of leucocytes ranges between 7,000 to 10,000, and from 35 to 55 per cent. of them are small mononuclears.†

There is a SLIGHT POST-OPERATIVE LEUCOCYTOSIS, a rise of from 5,000 to 10,000 cells, which is not due to infection.<sup>51 52</sup> The count reaches its maximum in at least twelve hours after the operation and is not accompanied by parallel changes in the temperature or the pulse rate. This leucocytosis continues not over thirty-six hours (some say five days). If, however, the count rises over 10,000 cells above what it was before operation (and this latter count should always be determined), and remains constant for over two days, one should suspect a post-operative pyogenic complication. The highest count

\* Barnes, Am. Jour. Insan., April, 1909, vol. lxxv.

† Cornell, Am. Jour. Insan., July, 1907, vol. lxxiv.

<sup>51</sup> Frazier and Halloway, Contrib. from the Wm. Pepper Lab. of Clin. Med., 1902, No. 3.

<sup>52</sup> Am. Jour. Med. Sci., September, 1902, vol. cxxiv.

was 26,300, six hours after the operation. The nature of the operation seemed to have little influence on the count, yet the height of the rise is roughly parallel to its extent and character. The highest count was in a nephrotomy, 32,000 cells. King found in no case a rise of 20,000. There is little relation between it and the post-operative fever. Chloroform anæsthesia can cause a true leucocytosis, but this is very transitory indeed; ether none.

In point of degree there is no sharp line between the leucocytosis of infected and non-infected wound repair, but the latter is on the wane at a time when the former is just beginning.

When a packing is changed the leucocytes may rise somewhat. In a closed wound the leucocytes are a good index of an infection.

The diseases causing, as a rule, no leucocytosis are *typhoid fever* (page 627), *measles* (page 622), and *tuberculosis* (page 624).

**Pseudoleucocytosis.**—Certain other blood-changes occur in much the same conditions and are supposed to have the same significance as a leucocytosis. Among these are iodophilia (page 567) and a relative increase of the polymorphonuclear neutrophiles while the total count does not rise above normal. This is seen in cancer, septicæmia, etc. Also degenerations of the leucocytes, fragmentation of the nuclei, as in cancer, the appearance of myelocytes, etc., have much the same significance.

**Leucocytosis of Malignant Tumors.**—Cases of carcinoma (page 640) and sarcoma (page 643), while not always, frequently present a leucocytosis. This bears no relation to the kind of tumor, except that it occurs more commonly with sarcomata than carcinomata. There is none in epithelioma of the skin, while in the case of some organs, for instance gastric carcinoma, it is common. On the whole it bears no relation to the situation of the tumor. The blood of a case of sarcoma has been described as even simulating a leukæmia. It is a leucocytosis of polymorphonuclear cells, and mononuclears in some cases as well, which disappears after the removal of the tumor. The occurrence of the leucocytosis in these cases is so variable that it certainly is not alone the presence, nature, or situation of the tumor which determines it.

**Post-Hemorrhagic Leucocytosis.**—After a large hemorrhage there is a rise of the leucocytes which begins in from ten to fifteen minutes, and in one hour reaches about 16,000 to 18,000. This lasts a few days and then disappears. It is an increase of the polymorphonuclear neutrophile cells. The cause cannot be a new production of cells, since it begins so suddenly; it is best explained by the tissue lymph which flows into the vessels in order to restore the volume of blood, carrying with it a large number of white cells. Many consider that the nature of the wound is important, since often with injury and without



hemorrhage there is a leucocytosis, while if the hemorrhage be the result of a very slight injury, as, for instance, that from a gastric ulcer, the duration of the leucocytosis is very brief, lasting in some instances but two days. Stassano and Billou<sup>53</sup> found that leucopenia followed severe hemorrhages; a true leucocytosis smaller hemorrhages.

In a case of cirrhosis of the liver with fatal hemorrhage from the stomach, before death the reds were 1,960,000, hæmoglobin 23 per cent., leucocytes 23,000.

**Agonal Leucocytosis.**—Belief in an agonal leucocytosis existed before the inflammatory leucocytoses were understood, and hence many cases may have been those of terminal pneumonia. Yet this does not explain all of the high counts at the last of a disease. Cabot's case, for instance, of pernicious anæmia resembled a leukæmia. Such cases are rare, it is true. In most diseases the leucocytes do not change or even drop at death, while in some cases they do rise, which Ehrlich ascribes to the slowing of the circulation and hence the accumulation of the leucocytes along the periphery of the blood-vessels. Arneth doubts an agonal leucocytosis, thinking the leucocytoses which occur then are easily explained by the disease causing death.

**Medicinal Leucocytosis.**—After the administration of any of a long list of drugs, including the ethereal oils, tonics, myrrh, turpentine, peppermint, whether by mouth or subcutaneously, there may result a considerable rise of the leucocytes. In the case of the drug by mouth it seems to be comparable to a digestion leucocytosis, while in the case of subcutaneous injection the local reaction also may be important. The list of these drugs is so long and varied that an enumeration is not valuable. It is interesting that the extracts of certain body tissues and organs seem positively chemotactic.

The reverse is also true, as in the case of blood poisons which destroy the cells, hence with phenacetin, the chlorates, and pyrogallallic acid there is even a drop.

**Other Causes.**—In the case of animals simple violence will cause a rise of the leucocytes. In man, hard work, severe sweat, heat and cold, will also; many vasomotor influences, slowing of the circulation, as for instance by cold, Thayer finding in typhoid fever that a cold bath, especially those leaving the patient shivering, would raise the count about 6000 cells; the formula remained the same.<sup>54</sup> In any cyanotic part the leucocytes are increased.

Violent exercise will cause leucocytosis, as was seen in the runners of a twenty-five mile race whose leucocytes rose from 14,000 to 22,000.<sup>55</sup>

<sup>53</sup> Compt.-rend. Soc. Biol., 55, p. 180.

<sup>54</sup> See also Becker, Deut. Arch. f. klin. Med., 1901

<sup>55</sup> Larrabee, Jour. of Med. Research, 1902, vol. ii.

**Mixed Leucocytosis.**—By this is meant an increase of amoeboid and non-amoeboid cells, that is, of granular and non-granular; but as commonly used it means the presence in a leucocytosis of neutrophile myelocytes. The best known condition is in leukæmia, in which the absolute number of myelocytes may be from 50,000 to 100,000. In no other condition does the absolute number of myelocytes rise above 1000 (Ehrlich). Leukæmia is the chief condition in which the eosinophile myelocytes are found, and where Mastzellen are increased. All forms of non-granular cells are also increased. The next most important condition is pernicious anæmia. Almost any leucocytosis could be called mixed, since the higher the count the younger are the forms which appear in the blood. In these cases myelocytes have no significance if they disappear as the count falls, but should they remain after the count sinks it means exhaustion of the bone-marrow (that is, if the septic features continue). The appearance of myelocytes in those conditions means more,<sup>56</sup> provided we grant that the leucopenia of such cases (typhoid fever, *c.g.*) is proof of the inhibiting action of the bacterial toxine on the marrow, which is by no means certain.

In cancer of the bone-marrow, sarcoma, and metastatic carcinoma, there is a mixed leucocytosis which some suppose is due to the negative chemotaxis of the tumor. In severe post-hemorrhagic anæmias and in various children's diseases, especially diphtheria, anæmia, rickets, congenital lues, and pneumonia just after the crisis, it also occurs.

**Mastzell Leucocytosis.**—The only condition in which these cells are increased in the blood is splenomyelogenous leukæmia, in which case they may be even 15 or 20 per cent. of the increased count. Isolated cases, as of cancer, septic bone disease, various skin diseases, and even chlorosis, have been reported.

**Increase in Large Mononuclears.**—The origin of these cells is unknown. They are increased absolutely in typhoid fever, post-febrile measles, and especially in malaria in which they are in large numbers, even 20 to 30 per cent. of an almost normal count, a point of diagnostic value.

**Lymphocytosis.**—Ehrlich classified increased leucocyte counts as active, passive, and mixed leucocytoses. A true leucocytosis is active because amoeboid cells are increased which are supposed to have wandered out into the circulation in response to a positive chemotactic agent. A lymphocytosis he called passive since he supposed these cells to be mechanically washed out of the lymphatic tissue.

That some lymphocytes are amoeboid on a rather hot stage (44° to 46° C.) is granted; that they can migrate into the tissues in certain skin diseases is also

<sup>56</sup> Schindler, Zeits. f. klin. Med., 1904, Bd. 54, p. 512.

granted; they may be the cells of a pleural exudate; yet there is little resemblance between the true leucocytosis and the lymphocytosis which would lead us to call the latter active. Rous \* found that in man a lymphocytosis follows active exercise. If, however, the exercise is severe (as in a twenty-five-mile run), this mononucleosis disappears, and there may be an absolute decrease in the number of mononuclear cells of the blood. In animals the count is increased during exercise by cells which enter the circulation through the peripheral vessels. The main increase is not due to the greater flow of lymph, since the increase of cells in the blood is even twice as great as that of lymph-flow through the thoracic duct and is a greater addition than any simple lymphagogue, as glucose, can produce.

Rous found that normally the lymph of the thoracic duct furnishes the blood with a large proportion of its lymphocytes, and that under similar conditions this supply is quite constant.

Physiologically a lymphocytosis exists in infants, and during a digestive leucocytosis. Pathologically it occurs in simple gastro-intestinal disturbances of children (page 636), in whooping-cough (page 556), in cervical adenitis, during the reaction to tuberculin, in malignant lymphomata, and in sarcoma multiplex cutis. The best illustration is lymphatic leukæmia, in which case even over 90 per cent. of the 140,000 cells may be mononuclears. There is an absolute increase in splenomyelogenous leukæmia, and after splenectomy there is very constantly a slow increase of lymphocytes to even twice the normal number, which begins in about a month and continues even one year.

The leucocyte count may be low and yet a true lymphocytosis exist, as in a recent case of amœbic dysentery, with a count of 2,500 cells, 68 per cent. of which were small mononuclears. The best illustrations among the chronic diseases are hereditary lues and severe rickets. The statement is usually made that in chlorosis, pernicious anæmia, debility, late typhoid, Graves's diseases, hæmophilia, scurvy, and during thyroid treatment there is a lymphocytosis, but in many of these cases the increase was only relative.

The leucocytosis of children is sometimes a marked lymphocytosis, as in the case with enlarged tonsils mentioned by Churchill, in which the count was 20,000, 70 per cent. of which were small mononuclears. In the diagnosis of lymphatic leukæmia these cases must be remembered.

Ehrlich mentioned an interesting case of general lymphosarcoma with but 0.6 per cent. of small mononuclears in the blood.

**Leucopenia** may result from the reduction in one group of cells or from a general reduction of all. The former is seen during typhoid fever. Below 5,000 cells per cubic millimetre is usually considered a leucopenia. Leucopenia is said to be the first step in a leucocytosis. In tuberculosis of lymph-glands the count is even below 600 cells.

\* Jour. of Exp. Med., 1908, vol. x; Proc. of the Soc. for Exp. Biol. and Med., 1907, vol. iv.

Cases of leucopenia have been reported under the name "alymphæmic lymphomatosis." For instance, a case (Schwartz) with fever and acutely swollen glands, had only 600 leucocytes per cubic millimetre, and they all lymphocytes (no autopsy). Türk said some of these cases show none of the features of an infection.

The relationship between abdominal troubles and leucopenia is interesting. Typhoid is a disease with leucopenia while limited to the intestine, with leucocytosis when other organs are involved; tuberculous peritonitis uniformly causes no leucocytosis; "abdominal influenza" causes no leucocytosis.

Following some fevers the count is low. At the end of typhoid it is often but 2000 cells. In a recent case of hæmoglobinuria the count of leucocytes ran nearly parallel to that of the red cells, with, at the lowest, 2,500,000 reds and 950 leucocytes.

In cases of starvation or malnutrition due to any cause the count is low; for example, voluntary starvation (page 553), or that due to disease as cancer of the œsophagus (page 648). In one of our cases of ulcerative colitis the patient's red count was 2,100,000, and the leucocytes 700 per cubic millimetre.

In acute miliary tuberculosis (page 626) the counts are very low sometimes, with a great reduction of young cells. In all chronic intoxications, alcohol, morphia, lead, ether, mercury, arsenic (hence the drop in leukæmia?), leucopenia is the rule.

**Eosinophilia.**—By eosinophilia is meant an absolute increase of the eosinophile cells. The average percentage in a normal case is from 2 to 4 per cent., and while often it is the percentage by which the eosinophilia is judged the term should be limited to those cases in which the absolute number of these cells is above 250 per cubic millimetre.

Those conditions in which these cells are increased vary so much that they are well termed the most capricious cell of the blood.

I. There is a **PHYSIOLOGICAL EOSINOPHILIA** during childhood.

II. **Diseases of the Hæmatopoietic Organs.** I. **BONE-MARROW.**—(a) *Splenomyelogenous leukæmia* is a diagnosis which Ehrlich says should be made only in case there is absolute increase of these cells. As a rule, they are much increased, even to 29,000 per cubic millimetre (Zappert). The number of undoubted cases, however, is increasing in which these cells are very few or, indeed, entirely absent.

(b). In sarcoma of the bone-marrow they are sometimes present in great numbers (page 643). In (c) osteomyelitis and (d) osteomalacia they are sometimes increased.

2. **SPLEEN.**—One year after extirpation of the spleen there slowly develops an eosinophilia which lasts for several months. These cells are increased to from thirty to fifty times their normal number, and may

constitute even 36.6 per cent. of the leucocytes. A somewhat similar condition is present in cases of chronically enlarged spleen, in which the percentage of these leucocytes may be from 7 to 12, and in new growths of the spleen. Possibly these spleens are functionless.

3. **LYMPH-GLANDS.**—That an eosinophilia may be due to disease of these glands is not certain. Cases of carcinoma have been cited, but metastases to bones have not been excluded. In one case with such metastases the eosinophile cells numbered 60,000.

III. **Asthma.**—In true bronchial asthma at the time of the paroxysm eosinophile cells may be from 10 to 20 per cent. of the leucocytes; in one case of Billings, between 53 and 54 per cent. This is of diagnostic importance in excluding asthmatic attacks due to other cause. In emphysema these cells are also increased, in one case being 53.6 per cent. of a total of 8300 leucocytes.

IV. **Skin Diseases.**—A large number of skin diseases are accompanied by eosinophilia. This depends more upon the extent of the lesion than its nature. The contents of the pustules are sometimes interesting, since all of the leucocytes are eosinophiles.

In one case of pemphigus reported by Zappert there were 4800 eosinophiles per cubic millimetre of blood; in one of pemphigus vegetans in this clinic on one day, with a total of 20,400 leucocytes, 2.6 per cent. were eosinophiles; on another day, 11.6 per cent.; in pellagra and psoriasis these cells are sometimes increased; in urticaria they may reach even 60 per cent. of the total number; in a case of purpura with cyanosis in this clinic, of 52,000 leucocytes, 11 per cent. and later, with almost as high a count, 25 per cent. were these; in certain cases of eczema they are increased; of two cases of scleroderma in this clinic, in one they constituted 2.4 per cent. of 7000 leucocytes; in the other, 3.3 per cent. of 10,500; in five cases of purpura simplex the red cells and hæmoglobin were practically normal; three had a leucocytosis of from 10,100 to 40,600 (eosinophiles, 12.1 per cent.). This last case was also one of myositis with the eosinophile cells running from 11.3 to 25.6 per cent. (total leucocytes 20,300), but no other evidence of trichinosis could be found. In one of three cases of purpura rheumatica there was a leucocytosis of 13,300. In three cases of Henoch's purpura the leucocytes in one were 10,000. In two cases of purpura hemorrhagica the leucocytes numbered 7500 and 6600; of the latter 4.5 per cent. were eosinophiles. In one case of purpura hemorrhagica 9.4 per cent. of the 5200 leucocytes were eosinophiles. In one case of morbus errorum the leucocytes were 5000, the eosinophiles 18 per cent.; they slowly diminished for four counts during one month when in the hospital, at which time they reached normal. After chemical irritation of the skin, for instance by mercuric chloride, these cells are much increased, even to 14 per cent.

V. **Parasites.**—Any parasite, from the harmless pin-worm to the most malignant uncinaria, may cause an eosinophilia. It is not always present, nor does its degree bear any relation to the severity of the infection or the danger of the parasite. Amberg in amoebic dysentery of children found a slight eosinophilia.

In trichinosis, Brown demonstrated this as a most important point in diagnosis, the maximum count being a total of 35,000 leucocytes, 68 per cent. of which were eosinophiles. This eosinophilia is not

always present, as in a case reported by Howard and another by Da Costa, but it was present in all the other 25 or 30 cases that have been reported. In Gwynn's case they were 65.9 per cent. In Brown's case they were high and fell gradually. The neutrophile cells are relatively and absolutely low. Brown was unable to get any Charcot-Leyden crystals from the blood, hence considers some other element than this necessary for their formation.

In uncinariasis the eosinophile cells have constituted usually from 8.2 to 10 per cent., in one case reaching 72 per cent. of the count. Our highest count was 13 per cent. in a total of 7400 leucocytes. In one case with *Tænia saginata* the eosinophile cells were 34 per cent.; of *Ascaris lumbricoides*, 19 per cent.; *Oxyuris*, 16 per cent.; *Strongyloides intestinalis*, 13.5 per cent.; *Bilharzia*, 20 per cent. In filariasis they vary from 4 to 17 per cent.; in Calvert's case, they were 22 per cent., and reached a maximum at day (in others at night). Calvert thinks that the number of eosinophile cells depends upon the acuteness of the attack, and so in long-standing cases there may be no increase. He found that the number of these cells in the circulation bears an inverse relation to the number of embryos, the former increasing during the day when the embryos disappear. In hydatid cysts of the liver an eosinophilia is considered important. It varies from 7 to 20 per cent., and in one case reached 40 per cent. During the afebrile stage of malaria these cells have risen to 20.4 per cent. In dracontiasis they are reported as from 6.4 to 36.6 per cent.

VI.—A **post-febrile eosinophilia** occurs after most fevers, or at least these cells increase to the upper limits of normal. In scarlet fever during the course these cells may vary from 8 to 15 per cent., but in all other fevers during the height they are diminished, and as the temperature drops they rise; in pneumonia, for example, they may rise to 5.7 per cent., absolute number, 430; in acute articular rheumatism, to 9.4 per cent., absolute number, 970; in malaria one day after the chill, to 20.34 per cent., or 1486; in varicella, to 16 per cent.; in measles, to 5 per cent.; and in rickets, to 20 per cent.

VII.—During a **positive tuberculin reaction** these cells may fall and then rise even to 26.9 per cent. (3220). In one case reported by Grawitz their absolute number was 41,000 of a total of 45,000.

VIII.—In diseases of the **genital organs** these cells are often increased; in all ovarian diseases except cancer; in ten of eighteen ovarian cysts and abscesses; and in gonorrhœa, especially posterior urethritis and prostatitis.

IX.—In **malignant disease** they sometimes constitute from 7 to 10 per cent. of the leucocytes, and in one case of lymphosarcoma they numbered 60,000 cells.

X.—After **certain medicines**, as camphor (to 9 per cent.) or the

inhalation of carbon dioxide they sometimes, though rarely, are increased.

#### XI.—In diseases of the sympathetic nervous system.

While the origin of an eosinophilia concerns the physiological pathologist rather than the clinical microscopist, yet we have considerable clinical evidence that these cells are increased in response to some specific positive chemotactic agent. The best recent review of this subject is that of Howard.\* We find in some pus, some sputa, etc., practically only eosinophiles, although the differential count of the circulating blood of these patients is normal.

It is important that in the blood of some rare cases we find a group of leucocytes which might be either neutrophiles or eosinophiles. (See page 500.) Were this the testimony of but few, their technic or their judgment might be suspected; but several have reported such cases.

**Iodophilia.**—By iodophilia is meant the presence in the blood of an unusual number of leucocytes, the protoplasm of which takes a brownish red color when the specimen is treated with iodine, or contains definite granules which take that color.

The reagent used contains: iodine, 1 gm.; potassium iodide, 3 gms.; water, 100 cc.; and gum arabic, 5 gms. A drop of this solution is placed on a slide, and an unfixed smear of fresh blood is pressed down into the drop. The excess of stain is removed with a piece of filter paper applied at the edge of the cover-glass, while this is held firmly against the slide.

In a smear of normal blood thus treated all the blood elements take a bright yellow stain. Under certain conditions, however, some of the polymorphonuclear neutrophile leucocytes contain granules (the "intracellular reaction") which stain brownish-red, or the protoplasm of which takes a diffuse brownish-red stain. Masses of granules similarly stained and from 2 to 8 microns in size are often found free in the plasma (the "extracellular reaction"). In judging the degree of iodophilia the number of the cells and the degree to which these are affected both are considered. Since all leucocytes contain some glycogen there is probably a degeneration of these leucocytes, perhaps of toxic origin, which increases their glycogen's affinity for iodine.

The reaction is positive in the greatest variety of conditions; in pernicious anæmia, in severe secondary anæmia, but not in chlorosis, and the moderate grades of anæmia; it is always positive in leukæmia. The number of cells bears a direct relation to the acuteness of the attack, and it is said has a prognostic value. La Franca found them in chlorosis, and considered only a large number of affected cells important. Locke considers it a test independent of, but of nearly equal value with, leucocytosis. It is positive in nearly all cases of septicæmia, especially those with leucocytosis, in cases with purulent

\* The Jour. of Med. Research, Dec., 1907.

exudates, hence especially pneumonia. It is invariably present in severe septic conditions (Locke).<sup>37</sup>

A clinical importance is claimed for the reaction in certain diseases, as in appendicitis. Locke found the intensity of reaction to depend on the severity and duration of the process in the appendix and the amount of septic absorption from the focus. But the point of greatest importance is, he claims, the occurrence of a marked iodine reaction without leucocytosis in some of the most virulent cases. After operation with free drainage the reaction disappears within forty-eight hours.

The pendulum has swung in regard to the value of this test, and many now condemn it as of little value surgically (Reich). Küttner gives it some prognostic value, an increase in intensity of the reaction being a bad sign.

The gynæcologists claim its presence to be of value in the diagnosis of pelvic abscess. In cases of ovarian cyst with twisted pedicle the test is negative, even though there is a high leucocytosis. The same is true of other conditions without pus formation.

**Blood Platelets.**—Blutplättchen, Plaques (Kemp, Osler), Hæmatoblasts (Hayem), (Plate II, 23). In the blood are the so-called "third corpuscles," small colorless bodies containing no hæmoglobin, about three microns in diameter, round, oval, or rod-shaped, according to the view-point, without a biconcavity, bluish, soft, homogeneous or granular, but not sticky and glistening when perfectly fresh as they are so soon after, which look and stain like nuclear material; they contain no nucleus, no membrane, and have in an ordinary fresh blood preparation a peculiar bluish refractivity like the protoplasm of a non-granular leucocyte. Platelets when perfectly fresh are slightly granular, but at once when removed from the blood-vessel become hyaline and glassy, then pale, and disappear, or unite to form a granular mass. Two important characteristics are to be emphasized. A platelet soon becomes a very sticky body, and unless special fixing fluids are used it attaches itself at once to the glass, to other corpuscles, or the platelets collect in masses of from two to hundreds. The other characteristic is its fragility, for it disintegrates rapidly, even in a few seconds. This disintegration is seen to best advantage in the masses of platelets, and the result is the so-called Schultze's "granular masses" (see Fig. 114), from the periphery of which radiate fibrin strands, and at the edges of which are vacuole-like areas, the so-called "viscous metamorphosis" of Eberth, or the "mucoid degeneration" of Osler.

Stained with the usual Romanowski mixtures, they are seen normally in groups of one to ten; they would seem to be composed of

<sup>37</sup> Jour. Med. Research, January, 1902.



nucleus and protoplasm, the nucleus consisting of rows of blue or reddish dots sometimes arranged in a spherical mass, the protoplasm-like substance sometimes hardly seen, sometimes swollen to give them almost the size of a red corpuscle.<sup>58</sup>

When the platelets rest on cells they resemble malaria parasites. It is to be noted that they have not the definite structure of the parasite, that they are surrounded by a clear zone from which the hæmoglobin has been pressed out by the platelet, while the protoplasm of the corpuscle comes up exactly to the parasite.

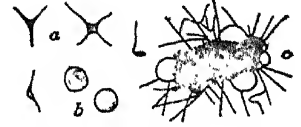


FIG. 114.—Platelets (copied from Osler): *a*, platelets in irregular shapes; *b*, with clear areas; "*c*," Schultze's granular mass.

Their size in the fresh specimens varies from 2.5 to 5 microns in diameter (Determann); 1.5 to 3.2 microns (Osler); 2 to 7 microns (Preisich and Heim). In general their size varies inversely as their number; that is, the more the platelets the smaller they are. Their fragility also is more marked when they are increased. Some soon show clear areas, either in the centre or on one side, or on the whole periphery; others become crescents, triangles, quadrangles, spindles, threads, etc. (see Fig. 114, *a*, *b*). It is much the best to study them at a temperature not over 40° F., for then these changes are much slower, requiring minutes instead of seconds.

It is customary to call anything a platelet which is smaller than a red blood-cell and which does not contain hæmoglobin. This is a mistake for the blood certainly contains cell detritus, and the term "platelet" should be reserved for bodies which have a peculiar bluish refractility, are very sticky, and which soon go to pieces. Anything floating in the plasma in a blood preparation to which a fixing fluid has not been added is probably not a platelet, although it may resemble it perfectly. Buds from red cells lose their hæmoglobin, become granular or glassy, "and cannot then be told from platelets;" "inner bodies" extruded from red cells, after undergoing certain degenerative changes, "cannot be told from (degenerated) platelets." One must decide whether he will term platelets all fragments which look like degenerated platelets. The question is simple when we are studying only perfect cells. It is difficult when we are counting degenerated platelets.

Specimens of the platelets are best obtained in the following manner: A drop of Picini's fluid (mercuric bichloride, 2; sodium chloride, 4; glycerin, 26; water, distilled, 226), or Hayem's fluid (water, 200 cc.; sodium chloride, 1 gm.; sodium sulphate, 5 gms.; potassium iodide solution [water, 100, potassium iodide, 5, iodine in excess], 35 cc.), or the fluid recommended by Kemp (0.9 per cent.

<sup>58</sup> See also Puchberger, Virchow's Arch., 1903, vol. clxxi. p. 181.

sodium chloride solution in 2.5 per cent. formalin), or Determann's fluid (distilled water, 160; glycerin, 30; sodium chloride, 1; sodium sulphate, 8; methyl violet, 0.025 parts). The best fluid, however, is a 10 per cent. sodium metaphosphate solution. A drop of the fluid is placed on the well-cleaned tip of the finger; the skin is then pricked through this drop so that the blood will at once mix with the fixing fluid; a drop is then placed on a slide and covered with a cover-glass.

To COUNT the platelets, the relation between them and the red blood-cells is counted in a fresh preparation. An ordinary red blood-count is then made, and from this the number of platelets per cubic millimetre may be easily calculated. Helber<sup>59</sup> counts them directly. The blood is quickly mixed with 10 per cent. sodium metaphosphate in a pipette giving a dilution of 1:30, and is then counted on a ruled slide similar to the ordinary counting-chamber, save that the thickness of blood-film is 0.02 mm.

The normal number per cubic millimetre has been found 250,000 (Osler); 225,000 (Determann); 245,000 (Enden). Yet the number varies much in the same person at different hours of the day, and in general the physiological variations are so considerable that only very great ones are to be considered clinically important. But Helber found no great daily variation (190,000 to 260,000; average 228,000. The remarkable closeness of these average figures is interesting). In the new-born for the first few days they are very few. In certain diseases they are very many; in other diseases very scarce. It is hard to classify these diseases, but most agree that they are increased in anæmias due to any cause, especially post-hemorrhagic, during the blood regeneration, and may be related to the red blood-cells as 1:10; the increase is surely a good sign; they are increased in chlorosis, decreased in pernicious anæmia and in any severe anæmia which is doing poorly. The greatest increase is in splenomyelogenous leukæmia, very low counts in lymphatic (Pratt). They are increased in chronic diseases with cachexia, and in conditions with malnutrition generally, the blood showing the marked changes of hydræmia, low specific gravity, poikilocytes, etc. The number of the platelets varies with the amount that the disease affects the blood, hence there are more in cancer and in nephritis than in the anæmia due to cardiac disease, tuberculosis, etc.

During acute fevers of long duration they are at first diminished, but increased during the third or fourth week as the patient begins to get weak. In typhoid fever a rapid diminution is considered a bad sign (Türk). In short sharp fevers there is at first a decrease, then a reactionary increase, the curve often resembling that of the leucocytes. The more acute, more severe, more threatening the disease, the higher

<sup>59</sup> Deutsch. Arch. f. klin. Med., 1904, Bd. 81, p. 316.

the temperature, the fewer the platelets, so that in malaria and pneumonia not one may be found. After the temperature drops, especially if by crisis, the platelets may rise above normal in twenty-four hours and continue so for two to three days, then return to normal. In erysipelas and septicæmia there is no preliminary decrease, but an increase from the start. In acute articular rheumatism there is a great increase.

Cases reported with total absence were a moribund case of pneumonia and one of nephritis, a case of pernicious anæmia and one of purpura.

Pratt<sup>60</sup> found no relation to exist between the coagulation time and platelet count.

The MEANING of the platelets has been much disputed. Donné considered them "globulins;" Schultze, fragments of broken-down leucocytes (also Howell); Bizzozero said they were independent corpuscles, a view which Dr. Osler also holds; Löwit said, artefacts; Hayem is the only one who considers them as very young red blood-cells. For several years the belief in these as independent corpuscles was held, until in 1897 Arnold taught that they were fragments constricted from red blood-cells or fragments of cells which had gone to pieces. Determann, following this work, pointed out that their number was usually increased in the same proportion that the red blood-cells showed signs of degeneration, that their fragility and their size depended on their number; he considered them merely a measure of the resistance of the red cells (Mosso). Schwalbe, Müller, *et al.*, consider this platelet formation from red cells a necessary preliminary step in coagulation.

Maximow<sup>61</sup> considers them the extruded inner body of the nucleoid. In specimens properly stained (Maximow used methylene blue and eosin, hence any of the modified Romanowski stains will do) all steps of this process can be found. Some cells have a blue-stained centre; in others this blue body projects from the cell; again it lies in a depression on the edge of the cell; others lie free. Sometimes the cell looks like a bomb bursting and discharging this mass. Engel thinks these masses are remnants of the nucleus, Maximow says not. Preisich is one of the last to insist that they are the extruded nuclei of the red blood-cells, hence are in constant process of formation; that animals with nucleated reds have no platelets; that platelets increase as the reds increase, and (this point is hardest to accept) that eosinophile leucocytes are white phagocytes which have ingested platelets.

On the other hand, platelets occur in greatest numbers where the polymorpho-nuclears are breaking down, as in splenomyelogenous leukæmia, and when a leucocytosis is subsiding; and least where these cells are the least numerous, as in pernicious anæmia, lymphatic leukæmia, *et al.*

The next work of importance is that of Deetjen,<sup>62</sup> who, using a special agar plate, considers that he has proved them independent cells, motile, and nucleated.

Deetjen's method is as follows: Five grammes of agar agar are boiled in 500 cc. of distilled water for thirty minutes to dissolve it, then filtered through a folded filter. To each 100 cc. of filtrate are added 0.6 gm. of sodium chloride, 6 to 8 cc. of 10 per cent. sodium metaphosphate ( $\text{NaPO}_3$ ), and 5 cc. of 10 per cent. acid potassium phosphate ( $\text{K}_2\text{HPO}_4$ ). After adding these salts the fluid should not be boiled nor much heated, lest the metaphosphate be converted to orthophosphate. The solution should now be clear. A drop is placed on a slide and allowed to cool.

<sup>60</sup> Jour. of Med. Research, August, 1903.

<sup>61</sup> Arch. f. Anatomie u. Physiologie, Anat., Abth. 1899.

<sup>62</sup> Virch. Arch., May, 1901, vol. clxii.

The most of the agar surface is cut away, leaving a smooth area about 2 mm. square. On this the blood-drop is placed and at once covered by a cover-glass.

Deetjen believes them to be actively amoeboid, the amoeboid motion requiring the above salts for its best demonstration. In such agar specimens they are round or elliptical disks, then in one or two minutes show a more refractive round inner body with a greenish tinge and a periphery of pale protoplasm which is "in active motion," changing shape so rapidly, and position as well, that it is hard to draw them. This may continue for hours; best on a warm stage. The stained specimens show the protoplasm and the inner body which takes a nuclear stain, and seems to consist of a chromatin net-work. The ease with which this can be seen depends on the amount they have "spread out." Some are larger than red blood-cells.

Wlassow was an especially severe critic of Deetjen's work, and pointed out that although platelets do change their shape they never resume their original one; they may extrude "pseudopods," but they never show true amoeboid motion; chloroform will stop the amoeboid motion of leucocytes, but not that of platelets; their nucleated structure is not proved; amoeboid motion is never seen in platelets in the circulation; Deetjen uses an agar field, which would much favor the production of diffusion currents; Wlassow, therefore, still believes them to arise in the red cells. Wlassow made quickly a fresh specimen of blood, then ran under the cover-glass a drop of one-fifth concentrated mercuric chloride solution; at once the reds become granular and a small refractive hyaline area appears, usually at the periphery. From this a bud develops which is sometimes an irregular mass of granules which increases and may become more thorny, and then separates from the corpuscle. This body may or may not contain hæmoglobin, those which do later lose their color and then cannot be distinguished from platelets. Others have confirmed Deetjen's work (Dekhuysen and Kopsch).

We have observed most of the above phenomena, but have seen no true amoeboid motion, that is, a change in position which the changes in shape will explain.

Kemp, in his recent interesting work on the blood at a high altitude, is confident some platelets do contain hæmoglobin, and hence is in doubt as to their origin.

The most important recent work is that of Wright, who by histological methods finds an independent origin for them in the bone marrow, and that of Cole,\* who showed that that serum which will agglutinate blood platelets will not agglutinate red corpuscles. This certainly is strong evidence against the existence of any genetic relation between platelets and red blood-cells.

**Reaction of the Blood.**—To litmus the blood is alkaline, and various methods have been proposed to determine the amount of this alkalinity. If by alkalinity is meant a preponderance of free OH-ions in the blood, the physical chemists have taught us that the blood is an almost neutral fluid, quite so when defibrinated, slightly alkaline when arterial, while the serum is almost as neutral as distilled water. But by alkalinity the clinical chemist means the acid-combining property of this fluid, or the amount of alkali in it which can be substituted by an acid. This is of interest and of considerable importance.

The reaction of the blood is due to the alkaline phosphates of sodium and the alkaline earths, and to the alkaloidal bases (Labbé). Yet chemically the blood acts as an acid from the presence of acid salts; that is, it contains unstable acid salts which react to color indicators as feeble bases, but which behave as acids, since, in the presence of true alkali, they become neutral. Brandenburg divides the alkaline components into two parts. The first is the diffusible alkali, the native,

\* Johns Hopkins Bulletin, 1907, p. 261.

or the mineral alkali, that is, the bases which are bound to carbon dioxide. This is diffusible, and may be measured by bringing the blood into contact through a diffusion membrane with alkaline fluids of various concentration until one is found which in contact with blood does not change its alkalinity. The other, and greater in amount, is the alkali- and acid-binding value of the proteids which varies directly with the amount of albumin, hence chiefly with the blood-count. The diffusible alkali is to the total as 1:5 in the case of the total normal blood; in the serum, as 1:2; in the corpuscles, as 1:8; and in cases of anæmia of various grades that of the total blood may vary from 1:3 to 6.

The alkaline tension (that is, the diffusible alkali) is rather constant, about 60 mg. of NaOH per 100 cc. of blood. The total alkalinity depends much on the blood-count, and hence is subject to great variations. The alkaline tension and the molecular concentration of the plasma run almost parallel.

The alkaline tension has been found reduced in diabetes mellitus, in uræmic coma, in pneumonia, and in certain cases of nephritis (small contracted kidney and acute toxic nephritis). These cases, then, may be said to show "acidosis," or evidence of acid intoxication.

**Determination of the Alkalinity.**—Over thirty-five methods of determining the alkalinity of the blood have been proposed, but not one has yet proved valuable.\*

**WRIGHT'S METHOD.**†—Blood is drawn from a finger into a capsule, and one or both ends of this are sealed. The blood is then allowed to stand from 3 to 24 hours, or until the serum has separated. When the blood has stood until a condition of constant alkalinity is reached, the serum is titrated. The titration is done by means of capillary pipettes like those used in the opsonin work. Solutions of normal sulphuric acid are diluted twenty fold, thirty fold, forty fold, fifty fold and sixty fold. The indicator is delicate red litmus paper, prepared by acidifying very fine (Grübler) neutral litmus paper in a solution of 1:20,000 hydrochloric acid, repeatedly washing it with distilled water, and then drying it in an oven at 120° C. It should be glazed or made as smooth as possible by rubbing it with a glass rod. Such paper should show alkaline reaction to normal-blood serum diluted forty times.

The acid solutions, serum, and indicator being ready, a pencil mark is made about 2 cm. from the end of the pipette to be used. Serum is drawn from the capsule into the pipette and almost up to the pencil mark, leaving an air-bubble index. Then an equal volume of  $\frac{N}{20}$  sulphuric acid is sucked in, and the serum and acid are mixed

\* Strouse, Johns Hopkins Hospital Bulletin, vol. xix, p. 139, May, 1908.

† Strouse, Johns Hopkins Hospital Bulletin, vol. xviii, p. 221, June-July, 1907.

by blowing them into a watch-glass (or, preferably, one of the cells of a water-color palette) and repeatedly blowing out and sucking in the fluid. If this proves acid when a drop of it is tested on a strip of the litmus paper, the mixing is repeated with weaker acids until the fluid just gives an alkaline reaction. After this is reached, for instance with equal volumes of the serum and the  $\frac{N}{40}$  acid, then an intermediary solution of  $\frac{N}{35}$  acid is made from equal volumes of  $\frac{N}{30}$  and  $\frac{N}{40}$  acid, and the titration is repeated with this dilution. The acid dilution just neutralizing the serum is considered the measure of alkalinity, and the alkalinity is expressed in fractions of normal acid, for example,  $\frac{N}{35}$ ,  $\frac{N}{40}$ , etc. No attempt is made to titrate with acids varying by less than a fivefold dilution. Wright found that in a series of 13 normal persons the serum possessed an alkalinity varying from  $\frac{N}{30}$  to  $\frac{N}{45}$ , or an average of  $\frac{N}{35}$ . In the author's words, this means that the "alkalinity of blood under ordinary conditions of health is such that the addition of one volume of a normal acid, 35 times diluted, to an equal volume of serum, just suffices to deprive that serum of its power of bluing sensitive litmus paper."

Strouse used Wright's method on a long series of cases, employing a technic which was painstakingly careful. He found that its limits of error were so wide that it had little value. Those cases with alkalinity so lowered by acidosis that the change could be perceived by this method had at the same time clinical and urinary changes which made the condition obvious.

**LÖWY METHOD.**—This is still the best method for the total alkalinity of the blood. In a special flask, on the neck of which are marks indicating 45 and 50 cc., are measured 45 cc. of 0.25 ammonium oxalate. To this are added 5 cc. of blood removed by a hypodermic syringe from a vein of the forearm. The blood is therefore laked and will not coagulate, hence the alkalinity determined is that of the total blood. This fluid is then titrated with twenty-fifth-normal tartaric acid, lacmoid paper saturated with magnesium sulphate as indicator. By this method 100 cc. of blood contain from 400 to 600 mg. of NaOH.

**SALKOWSKI'S METHOD.**—Salkowski's method is certainly simple. A known amount of ammonium sulphate is added to the blood and the ammonia of the salt thus set free determined by Schlösing's method.

Under a bell-jar are placed 20 gms. of finely pulverized ammonium sulphate which have been dissolved in 20 cc. of water. In a receptacle above this are placed 10 cc. of fourth-normal sulphuric acid. One then pours into the lower dish 10 cc. of blood. The measuring-glass to be used for the blood is first washed in 1 per cent. sodium oxalate solution to prevent coagulation. The blood is mixed with the ammonium sulphate solution, and the whole covered at once with the bell-jar. In five or six days all of the ammonia set free from the ammonium sulphate will have been taken up by the sulphuric acid and its amount determined by titrating this. By this method the alkalinity has been found for men, 350 to 400 mg. of NaOH per 100 cc.; for women, 300 to 350 mg.

**Urea in the Blood.**—A very simple method given by Herter<sup>63</sup> is as follows: A carefully measured quantity of blood is treated with three to four times its volume of absolute alcohol and allowed to stand twenty-four hours. The filtrate and washings are evaporated to dryness at moderate temperature, the residue taken up again in absolute alcohol, and again filtered. The filtrate and washings are again evaporated to dryness, and the residue dissolved in a little water. The nitrogen of this solution is determined by the sodium hypobromite method exactly as in the urine. Other nitrogenous extractives of the blood, as creatin and lecithin, will also furnish a little nitrogen, but these are small in amount and are not wholly broken up, while urea is by far the most abundant.

**Anæmia.**—Grawitz defines anæmia as a deterioration of the blood qualitatively and quantitatively as regards one or all of its constituents—the plasma, the corpuscles, and the hæmoglobin. The term as generally used means a reduction per cubic millimetre of the red blood-cells either in number or in volume. But these cells are really a relatively unimportant part of the total blood, having, so far as we know, but one function, and that a relatively minor although indispensable one, of carrying oxygen to the tissues, while the plasma contains the constituents upon which depend the life and health of the body, including the red corpuscles. In the plasma are the raw materials from which each cell gets new material for its structure, the food and fuel which it may use, and the many and as yet unknown bodies for its defence.

By anæmia, properly speaking, we should mean a diminution in the total volume of undiluted blood. The proof of this is impossible. The reduction in volume does occur, as observations at the autopsy table show; in cases of emaciation, for instance, and with a practically normal blood-count. On the other hand, by dilution with tissue-lymph the blood after a hemorrhage will maintain its volume, while the dilution is manifested by the red blood-count. Lastly, in other cases, as of pernicious anæmia, there is evident diminution in volume of blood and of the corpuscles.

But the essence of an anæmia is a poverty in the plasma of the protoplasm-building bodies, that is, of its proteids. Some have considered that the presence of anæmia was best determined by measuring the amount of water in the plasma, which may be supposed to increase as its proteids decrease, but this has proved unsatisfactory, since a dilute plasma may mean only an increased total volume of blood, while in the severest anæmias (clinically) with low count the plasma may chemically be almost normal.

<sup>63</sup> Jour. of Exp. Med., vol. iv, p. 119.



From the practical point of view a diminution in the percentage of hæmoglobin is accepted as the most sensitive index we have of any deterioration of the blood, and a diminished red blood-cell count a sign of a slightly more advanced grade, but first in value.

By *oligocythæmia* or *hypocythæmia* is meant a relative diminution in the number of red blood-cells; that is, those of a unit volume of blood are absolutely diminished. This may be due either to an actual reduction in number of the cells in the body, or to an increased amount of plasma. By *oligochromæmia* is meant a diminution in the amount of hæmoglobin per unit volume of blood. By *color-index* is meant the percentage of hæmoglobin divided by the percentage of the red blood-cells, 5,000,000 cells considered as 100 per cent. (See page 530.)

By *oligæmia* is meant a diminished amount of blood in the body. This may be suspected, but cannot be proved. *Oligæmia serosa* is an oligæmia of diluted blood; *oligæmia sicca*, an oligæmia with blood qualitatively normal. *Hydræmia* means an increased percentage of water, and occurs whenever there is a diminished amount of albumin. *Polyplasmia* is an increase in the volume of the plasma, supposed to occur in chlorosis; *oligoplasma*, a decrease, which occurs in certain cardiac diseases. By *plethora vera*, an increase in the total volume of blood. This can only be suspected.

By the *hæmatopoietic organs* one usually means the organs forming red and white corpuscles; the bone-marrow, spleen, and lymph-glands. The bone-marrow certainly is the building-place for red corpuscles and many leucocytes; the spleen is active, perhaps, after a severe hemorrhage, but otherwise is probably unimportant so far as the red blood-cells are concerned; it is possibly important in the formation of leucocytes, and is quite probably the organ which removes the old cells; the function of the lymph-glands as hæmatopoietic organs is still in doubt.

The red blood-cells have, so far as we yet know, the one function of transporting the oxygen to the tissues, an indispensable but relatively minor function of the blood. The function of the leucocytes is still imperfectly known; in general, they are said to be important in immunity, in the protection of the body against invading organisms, or toxins; in nutrition, since by means of them a great deal of neutral fat is absorbed into the intestine; and by going to pieces they certainly raise the albumen content of the blood. The function of the platelets apart from coagulation is not well known. While these functions of the formed elements are very important, those of the plasma of the blood are far more so, and we study the former because they are as yet the only index of the condition of the latter. Yet in studying the causes of anæmia those organs which form the plasma must be considered the most important hæmatopoietic organs. These are especially the intestine, the liver, and the kidneys, although every organ modifies to some degree the composition of the plasma. It is in the intestinal wall that the plasma obtains its proteid content; in the liver that the excess of carbohydrate is removed, or more furnished when necessary, that the ashes of the body are transformed to urea, etc.; and it is in the kidneys that the most of the ashes are removed. Certain of the glands with internal secretions are also important in modifying the constitution of the blood. The pancreas furnishes the internal secretion necessary in sugar metabolism, while the importance of the thyroid and the adrenal is well known. Lastly, in the muscles themselves the blood is modified, since they remove certain food constituents and fuel, and give in return the ashes of



these bodies. It is thus seen that every organ of the body is a blood-building or blood-modifying organ, either adding to or subtracting from the plasma certain bodies, and that the blood will suffer from disease of any one of these organs but especially of the intestine, liver, and kidneys. In nearly all disease of the blood it is probably the plasma that suffers first, and in many of the anæmias the diminution either in amount or in value of the red blood-cells may be merely the expression of changes produced in the blood-building organs by the abnormal plasma. The corpuscles cannot stay normal long in an hydræmic plasma. In pernicious anæmia and other toxæmias the element of blood destruction is of course also very important.

The anæmias may be classified as *primary* and *secondary*. By primary is meant one for which an adequate cause cannot be assigned. Here are included chlorosis, the essential idiopathic anæmias, the simple primary and the pernicious, leukæmia, and pseudoleukæmia. By secondary anæmia is meant one for which the cause assigned seems adequate to explain the blood condition. Under these causes are grouped hemorrhage, blood poisons, malnutrition, increased albumin destruction, cachexia, and poor hygienic conditions. The above classification is purely clinical, not hæmatological, and the autopsy table not infrequently shows to be secondary an anæmia which was during life supposed to be primary. The blood pictures are by no means sufficiently distinct. Points of differential diagnosis are easy enough to tabulate, but rather hard to apply in an individual case unless marked, and yet the anæmias of known cause seldom resemble the pernicious variety and it is seldom one finds a case with the picture of secondary anæmia and the cause not known. With the clinical history, the physical examination, and the blood examination, the diagnosis is usually satisfactory, but not always.

By *hypoplastic anæmia* is meant one due to insufficient blood formation. By *consumptive anæmia* one due to increased blood destruction.

From the hæmatological point of view one separates the *chlorotic* and *pernicious* forms. These terms are used carelessly. By the former is meant a slight or no reduction in the number of red cells and a definite often considerable reduction of the hæmoglobin. In this list are nearly all the milder grades of secondary anæmias, the primary chlorosis and the leukæmias. By "pernicious" in this connection is meant a great reduction in both cells and hæmoglobin, equal or with the color-index higher than 1. Such cases are the primary pernicious and rare cases of almost any extreme form of secondary anæmia.

**Secondary Anæmia.**—A secondary anæmia is, from the point of view of the pathologist, one of which the cause, known or suspected, seems sufficient to explain the condition. But whatever the cause, the picture which the term brings to mind is that of a blood of which the hæmoglobin is more reduced than the count of reds, and the plasma hydræmic. The red cells are usually smaller, of lighter weight, while some are large and "waterlogged."

Cabot suggests a classification for the secondary anæmias which is useful. *Mild cases* are those with a normal count, but with the hæmoglobin diminished; specific gravity slightly lowered; the count of the red blood-cells normal, yet a good many of them of light-weight, *i.e.*, small and pale in appearance, are seen. A *moderate grade* is one with a normal count, but the reds show qualitative changes; degenerations, microcytes, poikilocytes, crenated cells; the cells stain abnormally; there is less tendency to rouleaux formation. *Severe cases* are those with both qualitative and quantitative changes; the count, however, is not much reduced except in the anæmias of childhood, after large hemorrhages, in malaria, and in acute septicæmia. *Very severe cases* are those with all of the above mentioned changes, and in addition evidences of degeneration and destruction of the cells; evidence of regeneration (nucleated reds) will also be present.

**BLOOD PICTURE.**—In secondary anæmia the blood may grossly be pale. The reds are less reduced than the hæmoglobin, and their *count* may be normal. In severe cases, however, there will be a great reduction; in v. Limbeck's for instance with recovery, 306,000. A reduction of 1,000,000 cells, Bezançon and Labbé consider a mild hypocythæmia, one of from 2,000,000 to 3,000,000, an intense, while if the cells are reduced to one million, an extreme hypocythæmia.

The reduction in *hæmoglobin* is the constant and most important feature, and the best index of the grade (yet see page 530). The color-index is lowest in cases due to cancer, hemorrhage, and gangrenous processes, yet in none of these cases is it quite so low as in chlorosis. On the other hand in cases with extreme oligocythæmia the body, if given sufficient time, seems to protect itself by increasing the color-index, that is, by the production of cells which in size or weight are normal or above normal. Some think that the high color-index of pernicious anæmia is itself not characteristic of the disease, but an expression of the low count, the body, because of the chronicity of the disease, having had time to thus protect itself, while in those cases of secondary anæmia with low count and low color-index the acute course prevents this protective measure. The specific gravity of the blood is low. The dried residue is reduced. This is especially true in the cancer cases. (In one case of cancer of the stomach with a count of 1,400,000, 15 per cent. Hb, the dried residue was only 9 per cent.)

**Morphologically**, the stained cells show a lack of hæmoglobin, and yet a good many are normal. In many the biconcavity is very evident, and pessary forms are common. The polychromatophilic degeneration is common, is seen within twenty-four hours after a hemorrhage, but bears no relation to the hæmoglobin-content of the cell. The number of these basophilic cells runs fairly parallel to the grade of the anæmia.

so much so that this easy method has been suggested as a substitute for the more difficult of blood-counting (Walker).

*Poikilocytes* occur only in the severest cases, unless the term be used to include the variations in size, for the cells are much reduced, although unevenly so. Microcytes always occur, some even 2 microns in diameter. Large cells with "acute dropsy" have been described.

*Nucleated reds* vary much in number. This bears no relation to the anæmia, either its grade or its cause. They are abundant in some cases, as in acute post-hemorrhagic anæmia and in some chronic cases, while in others of the same degree they are absent. They may occur in crises (see page 543). The cells are normoblasts as a rule, microblasts occur in the severe post-hemorrhagic type, and megaloblasts are exceedingly rare except in cases of malaria and other diseases which affect the bone-marrow.

The *leucocytes* vary, depending on the cause of the anæmia and its complications, from a leucopenia to a leukæmic condition. Anæmia is a great stimulus to the bone-marrow, which during convalescence increases the count of the white blood-cells as well as of the red, hence, as a rule, there is a moderate leucocytosis with an increase in the polymorphonuclear neutrophils. In the other cases it is very hard to explain the picture. The *eosinophils* vary much, from few to an extreme eosinophilia. As a rule they are at the upper limits of normal, and if further increased some other reason than the simple anæmia is the cause.

The *platelets* are increased, even doubled in number. This is always true in the post-hemorrhagic cases.

**Acute Post-Hemorrhagic Anæmia.**—This anæmia may be acute or chronic. The loss of one-half to two-thirds the volume of blood at one time is fatal. Women tolerate hemorrhage better than men, and children least well of all.

The character of this anæmia depends upon the hemorrhage; whether the loss of blood occurred all at one time or at intervals. The clinical picture of these forms is very different.

The blood immediately after a hemorrhage is normal qualitatively, then, as the tissue-lymph is poured in to restore the volume, the count and the hæmoglobin diminish and the specific gravity becomes somewhat less since the lymph is richer in water than is the plasma. The color-index should remain "1" for a short time, then decrease, since the new cells hastily formed are "light weight," smaller in size, paler in color, and more easily degenerated, both as regards their shape and staining qualities. It is possible, of course, that some of these degenerated cells are old cells allowed to remain in the circulation longer than is normal, and that others are cells injured by the abnormal plasma.

The loss of from 50 to 70 cc. of blood even will cause an appreciable increase in water. The count falls steadily until the dilution is complete, and then as the new cells appear the count slowly returns to normal, the hæmoglobin somewhat later, it being weeks before the imperfect cells are entirely removed from the blood. The platelets are increased. The maximum hydræmia after one hemorrhage and the minimum color-index are on about the ninth day. There is often a post-hemorrhagic leucocytosis. The regeneration of the red blood-cells is rapid at first, and then slower. This early rapid increase some think due to division of the red blood-cells of the circulation, and in favor of this is the number of the small cells and of poikilocytes which are so soon found.

Nucleated reds may appear sometimes in large numbers, and disappear in about one week. They are chiefly normoblasts. Regeneration sometimes progresses in "steps" with blood crises (see page 543). The number of nucleated reds is often related more to the acuteness of the hemorrhage than to its severity.

In one case 13.7 per cent. myelocytes were found, which disappeared in three days. In another case of very severe post-hemorrhagic anæmia the polymorphonuclears were free from granules.

An early feature in the regeneration is the production of many large cells, in some cases these being the chief element of the blood picture.

TIME FOR REGENERATION AFTER ONE HEMORRHAGE.—The table given by v. Limbeck is:

Blood loss of 4.5 per cent. of body weight, thirty days to restore loss.

Blood loss of 4 per cent. of body weight, twenty days to restore loss.

Blood loss of 3 per cent. of body weight, ten days to restore loss.

Blood loss of 2 per cent. of body weight, eight days to restore loss.

But this varies with the age, nutritional condition, diet, and therapeutic measures. Grawitz says a loss of 3 to 4 per cent. of body weight requires fourteen to thirty days; of 1 to 3 per cent., five to fourteen days; a slight loss, two to five days.

Regeneration is quickest in men between twenty and forty years of age; slower in women, and slowest in children. After the regeneration is complete there may be even a hypercythæmia. Mikulicz stated that it was unwise to operate in cases in which the hæmoglobin was already or probably would be 30 per cent. after the operation. In our cases, however, we have operated with the hæmoglobin lower with good results.

In animals regeneration may be almost entirely prevented by feeding an iron-poor diet, especially if by previous hemorrhages the iron reserve supply of the body has been exhausted—a very suggestive point in human pathology.

In a case with repeated hemorrhages following abortion (produced evidently by some drug, not by an operation) the count on admission was 1,108,000; hæmoglobin, 18 per cent.; leucocytes, 4625; temperature normal.

In a case of hemorrhage from a badly crushed arm and after infusion the red cells fell in thirty-six hours from 5,000,000 to 3,000,000, and the hæmoglobin from 70 to 50 per cent. In a case of metrorrhagia the hæmoglobin fell to 19 per cent., yet the patient recovered; another with two post-partum hemorrhages had two weeks later only 11 per cent. hæmoglobin, and yet recovered.

Among the causes of this acute anæmia are traumatic hemorrhage, tubal pregnancy, in which a rapid anæmia is a bad sign, abortion (see above), uterine submucous tumors, ulcers of duodenum and stomach, typhoid ulcers, phthisis, aneurisms, varicose veins of œsophagus, rectum, or legs, the hemorrhagic diatheses, and hemorrhagic pancreatitis.

A case of *purpura hæmorrhagica* of eight weeks' duration<sup>64</sup> was admitted with a count of 696,000; hæmoglobin, 17 per cent.; leucocytes, 4000 (small mononuclears, 75 per cent.). At death seven days later the red count was 483,000, no poikilocytosis (since too acute?), no nucleated reds, no eosinophiles.

Ewing mentions a case of three weeks' duration with repeated epistaxis and a red count of 456,000.

**Anæmia from Chronic Hemorrhage.**—The conditions which obtain here are very different from those following acute hemorrhage. By chronic hemorrhage is meant a succession of hemorrhages at such intervals that the patient cannot recover from the one before the next loss of blood occurs. If the intervals are long enough for complete regeneration the conditions are merely those of acute hemorrhage, and the amount of blood lost in the aggregate may be enormous, as was well seen in the former days when venesection was a common practice. Ehrlich mentions a Russian physician with pulmonary tuberculosis, who in six and a half months lost twenty kilos of blood—that is, four times the total amount,—and yet recovery was perfect. In case the intervals are shorter, even though the total amount of blood lost be relatively small, the results are more serious. A case with repeated epistaxis due to telangiectasis of the nasal mucosa was admitted here several times, once with red cells, 2,288,000; hæmoglobin, 18 per cent.; leucocytes, 2800. Scurvy, especially if with much hemorrhage, causes a secondary anæmia, the red count averaging from 3,000,000 to 4,000,000. In one severe case it was reported as low as 370,000. A leucocytosis is often present but is due to some complication, otherwise there is a leucopenia. In one case in this clinic the red cells were 2,200,000; hæmoglobin, 40 per cent.; leucocytes, 2850. Other cases

<sup>64</sup> Billings, Johns Hopkins Hosp. Bull., May, 1894.

follow hemorrhage from lungs, uterus, hemorrhoids, from intestinal ulcers, cancer of the stomach, intestinal parasites, cancers, etc. The severe anæmia from high and hidden piles is now attracting much attention.<sup>65</sup> After long anæmia the blood-building organs seem to lose their ability to regenerate the blood, and the picture becomes that of a primary anæmia, rapidly fatal and without any sign of regeneration. It is perhaps the poor nutrition of the blood-building organs resulting from the anæmia which results in the pathological direction of their activity or their entire loss of function. In other cases it is merely the result of the chronic disease causing the hemorrhage. It, therefore, takes much longer for the blood to regenerate; in one case of hemorrhoids with a count of 2,600,000 it required eight months to reach normal (Ehrlich).

In this form of anæmia the hydræmia is considerable, the specific gravity is low, and the dried residue considerably diminished. The red count is much diminished, even to 1,000,000 cells. The new reds are small and pale, and the index low, 0.5 or even 0.44. The nucleated reds are scanty, the platelets increased. Sometimes, but seldom, the picture is that of a pernicious anæmia. While it may be that the patients die before this blood picture can develop, yet the evidence is against this idea since the index usually falls progressively lower until death. The leucocytes are increased at first, but when the anæmia becomes very severe there is usually a leucopenia. It seems as if the loss of blood protein was the element upon which all other features depend.

**Blood Poisons.**—These may cause anæmia by shortening the life period of the individual corpuscles. But since there is normally a good recuperative power, the poison must be severe, or if slight continue for a long time, to have a marked result. Such poisons are produced in many infectious diseases, especially the septicæmias, scarlet fever and lues; chronic poisons, as lead, arsenic, and mercury; the toxins of intestinal parasites, as *Bothriocephalus latus*; the poison arising in the intestine as the result of decomposition of the intestinal contents and in constipation; and especially the toxine of malignant tumors. All these may cause anæmia.

The effect of these poisons is sometimes seen in the red blood-cells in the circulation; the various degenerations, and the hæmoglobinæmia (plasmolysis). Other toxins are thought not to injure the cells in the circulation, but to cause an increased activity on the part of the blood-destroying organs, the liver, the spleen, and the marrow, without any hæmoglobinæmia. One of the best illustrations of the effects of such a supposed toxine is hæmatochromatosis, with the deposition of so much iron-containing pigment. The probability is that there has been a chemical (plasmotropic) change in the protoplasm of the cells which singles them out thus for destruction. Some poisons are thought to be purely plasmotropic, as for instance, lead, the toxine of cancer, of certain bacteria, and of ptomaines. Others

<sup>65</sup> See Herrick, Jour. Am. Med. Assoc., September, 1902.

in small doses are plasmotropic, in larger doses plasmolytic; in other cases the anæmia is thought to depend on the great differences in resistance of the reds (Grawitz).

**Anæmia of Inanition; the Anæmia of the Poor.**—This form is considered by some as a simple primary anæmia, by others as a secondary anæmia but due to a variety of concurring factors the relative importance of which cannot be apportioned, such as poor food, lack of sunlight, bad air, worry, and overwork.

Starvation alone will not cause anæmia; that is, not qualitative changes in the blood, but animal experiments as well as clinical observations have shown that there is a true anæmia, that is a diminution in the total volume of blood which runs parallel to the loss of weight. The blood picture of anæmia begins with the regeneration, since with the improvement in condition the blood does not keep pace with the gain of the other organs, and hence is diluted. The blood of Cetti, who fasted ten days, showed a rise in the red blood-cells of one million, a slight fall in the hæmoglobin, while the leucocytes fell from 12,000 to 4200. Others (Grawitz) consider that in some cases there is not simple atrophy of the total blood, but a loss of albumin of the plasma, hence a true anæmia. This is more evident if the days of fasting are alternated with days of slight nourishment, since the partial restoration of volume graphically becomes apparent. This is well seen in typhoid fever during the fourth week, in which case there is a rapid fall in the blood-count.

Poor food is an important cause of chronic anæmia of the purely hypoplastic form (Immermann). This anæmia is of the purest type, since it is due to insufficiency of blood formation. It is not so much the quantity as the quality of the food which is of importance, and unfortunately for the poor the most important foods, those containing iron, are the most expensive. These cases are met with particularly in those European countries where the diet of the poor consists of bread, potatoes, and other cheap food of similar nature. In this country, where there is by no means such a large class of poor on such miserable diet, the trouble is not so much the quality of the food as its preparation, good meat and vegetables being rendered indigestible by preparation in the frying-pan. In addition to this must be included the hurry in eating and the insufficient mastication of the food, which is a common sin in all grades of society. Bunge's experiments have shown that a diet poor in iron causes anæmia in a growing child, and yet it cannot be the lack of iron alone, since even the poorest foods have sufficient of this metal to replace the actual loss on the part of the body. With a truly non-proteid diet the effect on the blood can be demonstrated at the end of six or eight days, the first effect being a slight hydræmia, later the changes in the red blood-cells, which are probably secondary to the former.



Those living in dark houses are very apt to be anæmic. This is not due alone to the LACK OF SUNLIGHT, since hæmoglobin is not exactly comparable to chlorophyll, as the illustrations given by Ehrlich show; the horses which for from ten to twenty-four years are kept at the bottom of mines in Germany without seeing sunlight have normal blood; the members of Nansen's Polar Expedition remained for one hundred and forty to one hundred and fifty days without sunlight, and yet were healthy since the other causes of anæmia were eliminated. Although sunlight may not be so important for the adult, yet it has been shown to be important for the growing organism (Schönenberger).

To live constantly in an atmosphere of BAD AIR also seems to predispose one to anæmia and an excess of carbon dioxide is cited as the real cause, and yet the real relation of this single factor to anæmia it is difficult to determine.

In review it may be said that these factors all combine to cause the anæmia of the poor, and yet of them all overwork and worry, with their serious influence upon digestion and the nervous system, are probably the most important; hence it is that "anæmia of the poor" is really a misnomer, for the rich also suffer from disturbances of the gastro-intestinal tract which render their food almost as little nourishing, and worry is perhaps more their lot than that of the poor; hence it is that anæmia is perhaps quite as common among them.

There is a group of cases we diagnosed as secondary anæmia for which no one cause can be assigned. The great majority were women; the red cells showed a mean of about 3,000,000 (2,100,000 to 3,900,000); hæmoglobin, 30 to 50 per cent.; leucocytes, about normal. Such cases improve rapidly in the ward.

As has been said, one of the most important hæmatopoietic organs is the intestinal wall, the source of supplies for the plasma, hence indirectly for the cells.

GASTRO-INTESTINAL DISTURBANCES are some of the most important causes of secondary anæmia, and perhaps of many cases in which the intestinal feature is overlooked.

In our cases of *severe diarrhœa* in men, in 60 per cent. the red count was not above 4,000,000; in women the counts ran higher. The real anæmia must have been more pronounced than this, for in some cases the blood was probably concentrated by the loss of fluid (one case with 7,900,000).

The leucocytes ran low (even to 2700 and 2500) in some cases, but above 10,000 in 30 per cent. of all.

Cabot mentions a case with 1,928,000 reds, another with 2,440,000 and 10 per cent. hæmoglobin.

In *chronic dysentery* the count is high or low. One case had



1,520,000 red cells, another 2,500,000. On the other hand, one (male) had 7,000,000 reds, 110 per cent. hæmoglobin, and 7000 leucocytes, and one (a woman) 6,300,000 reds.

In *chronic constipation* our cases showed normal or high counts, as would be expected.

Our cases of *dilated stomach* showed nothing abnormal as regards the leucocytes; for the most part the red count fell within normal limits, but four showed considerable anæmia (3,300,000, 2,400,000, 2,250,000 and 2,600,000). Those cases with the vomiting of large amounts of fluid should have a concentrated blood; all severe cases would be expected to show some anæmia of malnutrition.

*Acute gastritis* during the febrile period shows a slight leucocytosis, true of 70 per cent. of our cases of gastro-enteritis. A slight leucocytosis is also common in *chronic gastritis*, except the alcoholic form in which cases the counts may be quite low.

One case of *chronic dyspepsia* had a count of red cells, 1,960,000; hæmoglobin, 42 per cent. (index 1.1); leucocytes, 3600 (of which s. monos., 11.3 per cent.; l. monos. and tr., 0.3 per cent.; pmn. n., 85.6 per cent.; eos., 2.6; normoblasts, 2 per 100 leucocytes).

In *ulcerative colitis* counts below 3,000,000 are not rare.

In *amæbic dysentery* one would expect the count to be little affected since the intestinal lesion is so local, and severe anæmia is rare, yet in 24 per cent. there was a slight (4,000,000 to 4,500,000), and in 12 per cent. a more severe (2,200,000 to 4,000,000) anæmia. A leucocytosis was the rule (70 per cent. of cases) at some time during the disease, the highest count being 19,200. Fitcher<sup>66</sup> found the general average of forty-three cases about 10,000. In children Amberg found an eosinophilia.

**Anæmia of the Tropics.**—It is said that Europeans after a stay of some time in the Tropics seem anæmic. Some consider this only apparent, and due to the distribution of the blood. The presence of basophile granulations in the red blood-cells, seen soon after the arrival there, and which were first described as related to malaria, would seem to indicate an injury to these cells. There are several tropical diseases, important causes of anæmia, which only now are we beginning to understand. These may explain some of the above cases.

**Chronic Infectious Diseases.**—Of these there are three which are most potent causes of anæmia,—lues, tuberculosis, and leprosy. While the toxine of the disease may be the most important element, yet the nutritional condition, especially the condition of the gastro-intestinal canal, the lack of exercise, and hemorrhages must also be included.

There is a great difference in toxins; in acute miliary tuberculosis without cyanosis, one of the worst septicæmias, there is little trace of blood destruction (see page 626).

<sup>66</sup> Jour. Am. Med. Assoc., August 22, 1903.

Anæmia is a common result of *pus formation*, and is due both to the toxins from the pyogenic organisms and to absorption from the pus focus of breaking-down tissue, and probably also to the over-taxation of the blood-building organs. The same is true in diseases with chronic *exudate formation*. *Albuminuria* is frequently cited as the cause of anæmia, and yet the actual daily loss of proteid to the blood-plasma even in a severe case is very slight, and could easily be replaced by one good meal. The poor condition of the digestive canal of nephritics is also important, but surely there is some toxin which has a deleterious effect upon the blood, as well as it surely does on the rest of the body functions. Dieballe has found a definite relation between the albuminuria and the hydræmia.

*Spermatorrhæa*, *lactorrhæa*, and diseases of the respiratory organs with a *large amount of sputum* are further causes. Yet cases with chronic purulent exudate formation maintain their blood condition surprisingly well, considering the drain there is on the blood, as in cases of chronic bronchitis and tuberculous abscess (see page 626). In all such cases it is the plasma which suffers first, the red blood-cells second.

In cases with marasmus there is an atrophy of the total blood which may cover an anæmia, while in other cases the anæmia may be more apparent than real, since there is a dilution of the plasma. At this point also may be mentioned the dilution of the blood from the absorption of effusions or other retained fluids. On the whole, the regulation of the blood is simply wonderful; for instance, after the removal of even seven litres of ascitic fluid at one time and its rapid reaccumulation the blood will show very little evidence of this enormous flux of fluid through the blood-vessels.

**Fever** is stated to be an important cause of anæmia, and yet it is not the elevated temperature but the toxins which cause the rise which also destroy the red cells, as evidenced by the increased hydrobiliruria. Most important are those cases of chronic cryptic septicæmia which for weeks may present the picture of severe anæmia without any suspicion as to the true nature of the trouble. On the other hand, **acute infections** will cause a rapid fall in the blood-count, as for instance Grawitz's case of streptococcus septicæmia, in which in a little over one day the reds fell from normal to 300,000.

A recent case of arthritis of unknown cause, but with blood-cultures negative, had a count which fell to, red cells 976,000; hæmoglobin, 17 per cent.; leucocytes, 4600. He improved rapidly.

In **yellow fever** considerable anæmia is found, in one case the count being 2,604,000, in another 1,400,500 (Maurel).

Pneumonia, diphtheria, scarlet fever, typhoid, acute articular rheumatism, smallpox, septicæmia, and other acute infectious diseases may cause a severe anæmia. The reader is referred to the various sections

on these diseases. In all cases, for the first few days at least, there may be no diminution in the red blood-count, even a hypercythæmia due to the concentration of the blood, seen best in diphtheria and typhoid fever, and which may cover a real anæmia. The rapid fall in the count which comes during convalescence or at the time of the crisis, as in pneumonia, is probably more apparent than real, and due to dilution of the blood resulting from the general vasomotor relaxation at that time (Grawitz); but the toxine of the infecting organisms may also be important by causing hæmolysis.

In many cases there is a drop in the count, but the quantitative changes are remarkably slight; only in very severe cases are microcytes, macrocytes or poikilocytes present. Hydræmia is the rule, the loss of albumin running parallel to the severity of the disease, and in severe cases reaching even 6.25 gms. of residue to 100 cc. of blood.

**Intestinal Parasites.**—Of the intestinal parasites there are two which are very important causes of anæmia.

**UNCINARIA DUODENALIS ET AMERICANA.**—Historically this form of anæmia is most interesting, since the cases of miners' and tunnel diggers' anæmia due to this parasite were first rated as primary pernicious anæmia, at a time before the distinctive blood-features of the primary and secondary anæmias were understood; now it is claimed the picture can rarely simulate the pernicious type. This parasite occurs in many different countries and bids fair to prove to have been one of the most important causes of anæmia; it is now thought to be in this country the chief cause of the "anæmia of the South." Our one marked case during the past five years had a count of red cells of 2,424,000; hæmoglobin, 32 per cent.; leucocytes, 9700; eosinophiles, 5.6 per cent.; but in some epidemics the count falls below 1,000,000 cells.

The cause of the anæmia is disputed. That it resembles one due to hæmorrhage rather than to a toxine is seen from the small amount of iron in the liver, it being diminished even to one-quarter its normal amount, to the absence of a leucocytosis, and the very low color-index.

In our three cases of *STRONGYLOIDES INTESTINALIS* infection the blood showed: red cells 5,420,000, hæmoglobin, 82 per cent., leucocytes, 6200; 3,560,00, 57 per cent., 21,500; and hæmoglobin, 60 per cent., leucocytes, 7500, respectively.

**BOTHRIOCEPHALUS LATUS.**—This parasite is the cause of a most interesting anæmia. It is a tape-worm which may live for years in the intestine of a person whose blood is normal, and yet in other cases cause the most severe anæmia, the almost exact picture, both quantitatively and qualitatively, of the primary pernicious type, but which recovers after the worm has been expelled. In Lichtheim's case the red blood-corpuscles were 500,000; hæmoglobin, 20 per cent.; six

worms were expelled. In Schapiro's case the count was 837,000, and in twenty-three days after the worm was expelled, 2,975,000. Bezançon and Labbé give as the average of reds 1,300,000, and the limits from 395,000 to 2,150,000; those of the color-index 0.9 and 1.62. All the degenerations and other signs of a severe primary anæmia, poikilocytes, microcytes, macrocytes, the polychromatophilic degeneration, etc., are present. Even one-half the nucleated reds are megaloblasts, and yet in two weeks after the worm has been expelled the megaloblasts all disappear, and in three weeks the megalocytic blood returns to normal type, with even normoblasts gone. The leucocytes are normal both quantitatively and qualitatively.

The cause for this anæmia is unknown. Surely it is not loss of blood; it is not the presence of the worm alone, since but 16 per cent. of the persons thus infected are anæmic. It is not the duration of the infection, for some persons are hosts for even twenty years before the anæmia begins. Schaumann emphasizes the predisposition of the patient. Dehio says that only those worms which are diseased or dead cause anæmia. But the diseases of the worm are not always evident. There are cases with a diseased worm, but no anæmia, and in other cases after the worm is expelled the anæmia is not cured. In this anæmia the iron of the liver has been found even twice normal in amount, which would indicate an intravascular destruction of the red blood-cells. The color-index is above normal.

*Tænia saginata* and *Tænia soleum*, *Strongyloides intestinalis* (in cases of "diarrhoea of Cochin China" due to this parasite, counts as low as 760,000 have been reported), and *Ascaris lumbricoides*, are sometimes, it is claimed, causes of anæmia.

**Yeasts.**—In four cases of systemic blastomycosis a moderate secondary anæmia has been found present (*e.g.*, 3,992,000 red corpuscles per cm.), and in eight cases a leucocytosis varying from 9,600 to 21,200. In two of these cases this yeast was isolated in pure culture from the blood.\* In one case of infection with *Oidium coccidioides* there was a leucocytosis of 17,000.†

**Poisons.**—Lead, mercury, arsenic, certain organic poisons, plant and animal toxines, ptomaines, and the toxines of burns, all may cause anæmia. Lead is an especially potent cause, both of the acute and chronic forms. While lead causes essentially a chlorotic anæmia, manifested first by the degenerations of, and not by diminution in the count of the red blood-cells, it may be so severe that they are reduced to even 1,300,000. Megaloblasts sometimes are found. The basophile granules are very common and important (see page 511).

We have had 17 cases of anæmia due to lead. In 16 cases the lowest was 2,900,000; in 7 cases the red count was over 4,500,000; the mean, 4,200,000. Hæmoglobin, lowest, 38 per cent.; mean, 60 per cent. In 10 of 16 cases the leucocytes were above 10,000, maximum 25,000, but fell very soon after admission.

\* Montgomery and Ormsby, *Arch. of Int. Med.*, vol. ii, No. 1, p. 1, Aug., 1908.

† Hektoen, *Jour. of A. M. A.*, Sept. 28, 1907, vol. xlix, p. 1071.

Long-continued use of certain of the coal-tar products causes a severe anæmia. Stengel and White<sup>67</sup> report a most interesting case, a woman with reds 2,092,000; hæmoglobin, 35 per cent.; leucocytes, 19,800 (a previous count), and 32,323 nucleated reds per cubic millimetre, of which 91.4 per cent were normoblasts, 3.5 per cent. megaloblasts and 5.3 per cent. free nuclei. The platelets were increased. There were many poikilocytes, a few basophile granules, and considerable polychromatophilic degeneration. It is interesting that the diagnosis of this poisoning was made from the appearance of the smear alone, despite the repeated assertions of the woman of the impossibility. It was found to follow the use of acetanilide. They mention Ehrlich and Lindenthal's case with nucleated reds in the proportion of 1 : 56 of the red cells. In Brown's case of acetanilide poisoning<sup>68</sup> at death the reds were 1,166,000, and the nucleated reds 22,150 per cu. m.m.

**Splenic Anæmia** is the name given to a group of cases with anæmia and idiopathic enlargement of the spleen. The anæmia is of the secondary type, the average of Osler's cases being over 3,000,000; there is no leucocytosis, or a reduced count. Such cases have profuse hemorrhage from the stomach and œsophageal varices. In one case in Osler's series the macrocytes and gigantoblasts were a marked feature of the case.<sup>69</sup>

**Simple Primary Anæmia.**—This form, which some separate from primary pernicious anæmia because of the differences in the clinical course, is also hard to separate from those secondary anæmias already mentioned as due to unhygienic conditions, poor food, hard work, worry, etc. It is a severe primary anæmia, characterized by the number of relapses, ending finally, however, in death. This type can be recognized only when the case is typical. It seems to stand midway between chlorosis and primary pernicious anæmia, some cases differing from the former only in the age of the patient, others presenting many features of the latter, and between them every gradation. Midway between these extremes is a group of cases with oligocythæmia and oligochromæmia of about equal grade, and leucocytes normal both quantitatively and qualitatively.

**Progressive Pernicious Anæmia.**—Eichorst's definition of this was a severe anæmia which in spite of all treatment progresses relentlessly to death. Pathologically, there is no lesion of etiology. The blood picture alone is not characteristic, for several varieties of secondary anæmia may assume a somewhat similar picture—"secondary pernicious anæmia." And yet the blood picture is so striking that the word "primary pernicious" now carries with it an idea of the blood picture

<sup>67</sup> Contrib. of the Wm. Pepper Laboratory of Clinical Medicine, 1903, No. 4.

<sup>68</sup> Amer. Jour. Med. Sci., 1901, vol. cxxi.

<sup>69</sup> Osler, Am. Jour. Med. Sci., January, 1900.

as well as its clinical and pathological significance. As illustrations of secondary pernicious anæmia are certain cases of cancer, phthisis, lues, malaria, repeated hemorrhage, lead poisoning, certain parasites, lesions of the bone-marrow, especially tumors, also osteomyelitis, atrophy of the gastric mucosa, stenosis of the pylorus, nephritis, certain rare cases of pregnancy, and purpura hæmorrhagica. In all the above cases there is a long history of anæmia-producing agencies, and this picture may represent the final stage, an almost complete bankruptcy of the blood-building functions.

The salient characteristics of the blood of primary pernicious anæmia are: Signs of rapid blood destruction (the degenerated reds, endoglobular degenerations, polychromatophilia, the urobilinuria, jaundice, the increased iron compounds (?) in the serum and the corpuscles, and the increase of iron stored in the liver and spleen); the poikilocytosis, a high color-index, and the megaloblastic blood formation. At first the poikilocytosis was supposed to be characteristic (Quincke); this idea was very soon corrected. Then the high color-index (Laache and Kahler) was supposed to be the important feature, and this is now the opinion of many. A high-color index may occur in severe cases of secondary anæmias, but it occurs in mild primary cases. Ehrlich considered that megaloblasts were characteristic, but this also is not strictly true, although they are numerous here.

**Volume of the Blood.**—Clinically there is no way of determining the volume of the blood, yet from the appearance of the patient we are often sure it is diminished, and at the autopsy table are sometimes astonished at the small amount of blood in the heart and blood-vessels. A remarkable case was seen by the writer in Professor Müller's clinic, in which all the organs seemed almost exsanguine.

**Gross Appearances.**—The ear is a better place to obtain the drop of blood than is the finger. It may flow freely, or it may be difficult to get any. Lazarus considers that the former occurs when the patient is doing badly, and that the latter is evidence of improvement.

The blood is pale, of a light red watery color (*Fleischwasser*), and does not at all resemble blood.

We showed a tube full of this blood to a class on one occasion, asking them to tell from its appearance alone what fluid it was, and many of them said it was a cloudy urine, which, indeed, it did resemble.

The drop of blood is often streaked, evidence that the corpuscles have collected in masses. Cases have been described in which it is grossly of a coffee-color, probably due to hæmoglobinæmia. The coagulation time is often increased.

**Red Blood-Cells.**—In the fresh specimen these are seen to be few in number, and there is absence of rouleaux formation. The cells vary

much in size: many are slightly above normal, some very large; many are small, some very small. Only a few of the cells show Maragliano's endoglobular degeneration, but many do show another degeneration, the accumulation of the hæmoglobin in the centre of the cell. The most are of a uniform dark color. Nucleated reds will often be found in the fresh specimen. In a well-marked case the appearance of the fresh specimen alone will strongly suggest this disease. One has only to compare it with a specimen of normal blood, and the difference is striking.

COUNT.—An extreme oligocythæmia is the rule, and it is remarkable how few symptoms accompany these low counts, particularly as the volume of blood is also diminished. On the first visit the average cases will show a count of about 1,000,000 cells. Cabot's average was 1,200,000.

In our cases in the 102 admissions (several of the 81 cases being admitted more than once) the average first blood-count was 1,575,000. This is somewhat higher than that which other observers have reported, and is due to the fact that we had several cases admitted not for the symptoms of pernicious anæmia alone, but from attendant conditions, for instance, for nervous disorders. In 81 per cent. of our cases the count on admission was under 2,000,000 and in 12 per cent. under 1,000,000.

A man with a count as low as 500,000 may remain comfortable and active, while others with four times that number of cells suffer. Evidently the reason for the symptoms is not the oligocythæmia alone. Cabot thinks that the counts tend to remain at about 1,000,000 cells, dropping rapidly to this point and remaining there, then sometimes in improvement gaining rapidly to about 3,000,000; later to return to about this same figure. The count may remain stationary for some time, or it may diminish progressively until death. Quinke reported one case with a blood-count of 143,000, and yet who recovered. Hayem reported a fatal case whose lowest count was 292,000. Scott's case had at death 268,000 reds; index, 2; leucocytes, 5900.<sup>70</sup>

After admission the count may continue to drop for a while, then to rise, or it rises at once, or it remains stationary.

It cannot be too often emphasized that a change in count may mean a change in the total number of red cells or a change in the volume of plasma.

An interesting fact already noted is that clinical symptoms seem to bear no relation to the red blood-cell count. Certain cases enter the hospital with a few symptoms and a blood-count of 1,500,000, while other cases are apparently in no worse condition and yet have a count below 1,000,000. The comparative comfort and physical strength of such patients is in marked contrast to cases of chlorosis and the secondary anæmias, which cases enter the hospital with the blood in an apparently much better condition. Again, in some of those cases in which the blood continues

<sup>70</sup> Am. Jour. Med. Sci., 1903, vol. cxxv. p. 397.



to fall after admission and then to rise, it is curious that the patient feels so well that he insists upon going home at a time when the count is no higher or very little higher than on his admission. In other cases in which the count rises after admission and then falls, death occurs when the count has reached the level of admission. In still other cases with an initial drop, as in five of our series, the count was rising at the time of death.

Although patients come to the hospital for symptoms which bear little relation to their blood-count, yet the same case on two or more admissions will enter with counts which are curiously close.

The red blood-count on the day of death in two of our cases was high—2,700,000 and 2,100,000; in three cases moderate, 1,031,000, 1,326,000, 1,216,000; and in thirteen cases, and this we think a hint of the blood picture at death due to this anæmia alone, the count was between 718,400 and 376,000, an average of 567,700.

The blood during intermissions is not quite normal. The red count is about 3,000,000, and the cells still large (Cabot). The color-index, however, is sometimes low, and the leucocytes increased by an increase in polymorphonuclears; nucleated cells disappear. The diagnosis now is important, especially to insurance examiners. In a recent case with almost normal blood the diagnosis was made by one examiner and the case refused. He succeeded in getting heavy insurance in another company, and died in about one year of this disease.

The VOLUME of the red blood-cells is best determined by sedimentation. The average volume, instead of the normal 45 to 50 per cent., is from 8 to 10 per cent., which seems high, considering the count, and is a measure of the large size of the cells. Capps found the volume index always above the color-index, hence the size of the cells explains satisfactorily the latter.

SIZE.—The average diameter of the red cells is somewhat increased, with, however, wide variations, the cells measuring from 4 to 13 microns, and with extremes beyond these. The average diameter may be 9 microns. In no other disease are there so many macrocytes. It is not the average but the mean size, or the percentage of macrocytes, which is of importance in diagnosis, since the microcytes will lower the average.

MACROCYTES.—Seventy per cent. of the cells may be very large, between 11 and 13 microns in diameter (Lazarus). In a case reported by Ewing 90 per cent. measured from 11 to 16 microns. Gigantocytes also occur. These large cells are less biconcave than normal, some are not biconcave at all. Some are oval; some seem flabby; they are often dark colored in the fresh, often polychromatophilic in the stained specimens; they never present the pessary form; some, however, are pale, according to Grawitz many are, but the dark color of many of the large cells is, we consider, a quite constant feature, and one recognized by students when studying the fresh blood of patients concerning whom they know nothing. Students should be given many such specimens to study, always making a specimen from normal blood for



comparison, until they will say unhesitatingly whether the size of the average cell is increased or not, and whether the color is darker or lighter than normal. The differences are more striking than would be expected by one not trained to observe them. In some cells there is a slight change of color shade as well as of depth. There are cases in which the blood is said not to be megalocytic. Macrocytes are not common in normal bone-marrow, and their presence here is considered (Laache) a compensatory attempt to replace the amount of hæmoglobin-containing protoplasm. Cohnheim first said it was reversion to the embryonic type. Ehrlich attributes it to a megaloblastic degeneration of the bone-marrow.

**MICROCYTES.**—These cells vary from 2 to 6 microns in diameter, and are usually of a deep color. They occur in large numbers also in secondary anæmias. They may fail here. So numerous are these cells in some cases that the average size of the red blood-cells is not above normal; hence the importance of judging the mean rather than the average size. The dark color of these microcytes may be due to their spherical shape, but they have sometimes a greenish tint, which would indicate a chemical change in the protoplasm. These microcytes might be suspected to possess amoeboid motion, at least they change their shape and move quite actively among the other cells with an oscillatory motion. They have been described as monads, a leptothrix form, bacteria, and Hayem called them pseudo-parasites. It is very interesting to watch them in their movements. Such cells are pictured in Fig. 93.

**POIKILOCYTES**, the presence of which was supposed formerly to be a characteristic feature of the blood in primary anæmia, occur often in large numbers and in a great variety of shapes. Hook, raquette, spindle, and various dwarf forms occur. The sausage and the battle-dore shapes were formerly supposed to be found only here. The small forms show interesting contraction phenomena resembling amoeboid motion (see above).

It is not unusual to find shadows among the deep-colored red cells. Since in a rather acute case abnormal cells did not appear until about two months from onset, McCrae suggests that such cells occur only after the condition has existed for some time.

**POLYCHROMATOPHILIC DEGENERATION.**—This is best studied in pernicious anæmia. Although it is not always a sign of degeneration, in this disease for the most part it seems to be. With Ehrlich's triple stain these cells are a pale gray (Plate I, 25-28). With methylene blue they take a blue tint. Their number is almost parallel to the severity of the case (Grawitz). Red cells with Grawitz's basophile granules are very common, especially in severe cases, and have, Grawitz thinks, an important prognostic value.

**Nucleated Reds. NORMOBLASTS** (Plate I, 29, 30, 35, and Fig. 113,

a, c, d, e).—These cells, described on page 512, occur quite constantly in pernicious anæmia, alone or with megaloblasts, and in especially large numbers during the blood crises. In a case of Bezançon and Labbé there were from 6000 to 10,000 normoblasts and 960 megaloblasts per cubic millimetre. Many of these cells show polychromatophilic degeneration, especially those in which the nucleus is dividing.

The *blood crisis*, so interesting a feature in cases of severe anæmia (see pages 543 and 596), is not always, as v. Noorden thought, the sign of a regeneration active enough to be followed by a jump in the red count, although in secondary anæmia and chlorosis this may be the case. The crises probably do indicate an attempt of the bone-marrow to replenish the blood, but in some cases of pernicious anæmia they are followed by a fall in the red count, the convulsive attempt to stem the tide of destruction proving futile. Those followed by improvement occur especially in younger persons.

In some cases there are few or no nucleated reds in the peripheral blood. This means a slower regeneration. In other cases just before death all these cells disappear.

MEGALOBLASTS (Plate I, 32, 33, 38, and Fig. 113, f).—These cells were first described by Ehrlich as characteristic of pernicious anæmia. They may, however, occur in any anæmia, and in any disease involving the bone-marrow. The difficulty in forming a judgment concerning their occurrence lies in the fact that cells which one man counts as megaloblasts others do not. Many criteria have been proposed for their recognition—the size of the cell, the size of the nucleus, the structure of the nucleus, etc. Since the presence of these cells is so important in diagnosis it is safer when in doubt about a cell not to call it a megaloblast. One sees large cells with nuclei like those of normoblasts and small cells with nuclei like those of megaloblasts. Both of these groups we count as intermediate cells, and reserve the term megaloblast for a large cell whose nucleus is about the size of a normal red blood-cell; that is, about 7 microns. These cells are round or oval; they vary from about 11 to 20 microns in diameter. When very large they are called gigantoblasts. They are plump, often diffuent, and polychromatophilic. The nucleus in the fresh specimen has a well-defined chromatin net-work, but takes with the Ehrlich stain a pale greenish tint, staining so faintly that it may be overlooked; it is large, plump, round or oval, especially the latter; it is often surrounded by a clear circle, and outside of this circle the protoplasm often stains deepest; karyokinetic figures are sometimes seen, to find which some consider a grave sign. It is no easy matter to tell a polychromatophilic megaloblast with a palely staining nucleus from some mononuclear leucocytes, and, strange as it may seem, sometimes men of recognized authority differ whether to call a stained cell white or red (Plate I, 36). Color.

in a stained specimen, counts but little; the opacity of the protoplasm counts much, the red cell being more opaque. The point upon which most depends is the edge of the cell, for the spherical leucocytes must flatten out in the preparation, and so have a thin frayed margin, while red blood-cells, being disks, do not flatten, and have a thick, smooth, uniform, rounded edge, best seen when one cell touches another. Again, the edge of a leucocyte may overlap a neighboring cell, but the edge of the red cell merely flattens against it. There is no staining reaction which is characteristic for hæmoglobin, especially when basophilic, and one can be sure whether or not a cell contains hæmoglobin only when he sees it in the fresh state. A cell concerning which one is doubtful is more probably a leucocyte than a megaloblast, at least that is the safer view to take.

Although megaloblasts occur most commonly in this anæmia they are nevertheless rare here, and even Ehrlich, it is said, would hunt for hours until he found this much desired cell upon which he would stake the diagnosis of an otherwise clear case. If after a long search one finds six or eight such cells, he should be more than satisfied, and in some cases none will be found at times, and many on a later occasion. Their presence in large numbers is rare and ominous. Their number varies from day to day, there being interims of improvement during which they are absent, reappearing during a relapse. In other cases, however, they have been found during these periods of intermission while the blood-count is fairly normal. It is sometimes necessary to hunt two hours for one such cell, and yet the importance of this cell justifies that trouble. Upon the percentage of these rests some prognostic value. In megaloblasts polychromatophilia and the basophile granulation are particularly well marked.

Typical megaloblasts are found most frequently in pernicious anæmia, even in the mild grades, and for the diagnosis of these cases they are of great value. They occur rarely in other anæmias, except of children. They are present in bothriocephalus anæmia; a few are found in cancer cases, especially those with metastases to bone-marrow. We have seen as beautiful ones as could be desired in simple tertian malaria without any marked anæmia, and they occur perhaps always in malaria of children. They occur in large numbers in splenomyelogenous leukæmia; in secondary anæmias and chlorosis very rarely. They would seem to occur especially in those conditions in which the bone-marrow is involved, and of these, apart from the anæmias, malaria is a good illustration. Some consider their presence an indication of the severity of an anæmia, not of its form; others that it is a sign of bone-marrow involvement (by infection, new growth, etc.); others consider that large cells express an effort to increase the volume of hæmoglobin-carrying protoplasm. Another explanation suggested for

megaloblasts is that they are swollen hydræmic cells, that is, are dropsical, and contain the increased water of the plasma. It is true that some, the so-called "chlorotic cells" do suggest this very strongly, but they are always paler in tint; in cases of marked hydræmia there are often no such cells present, as, for instance, in nephritis, and they also occur in large numbers in chlorosis, and there the plasma is practically normal.

INTERMEDIATE FORMS (Plate I, 31, 37, and Fig. 113, b).—This is a most troublesome term. In some cases this group includes nearly all of the nucleated reds found in the blood. We have made it an invariable rule that all cells suggesting megaloblasts and concerning which there is any doubt shall be put into this group. That is, a nucleated cell the size of a normoblast with the nucleus of a megaloblast, or a cell the size of a megaloblast with the nucleus of a normoblast, is assigned here, hence the group contains a great variety of sizes both of cells and nuclei. It is very evident to one studying the pictures of the blood cells of cases reported as pernicious anæmia that there is wide divergence in the use of the term megaloblast. This accounts for many contradictory statements as to their frequency.

Whether these cells are intermediate between megaloblasts and normoblasts we do not know; by the term we merely mean a cell concerning which we are in doubt. The existence of transitional cells has been denied by Ehrlich and Pappenheim. All transitions have been found by others (Schaumann). These occur in conditions in which megaloblasts would be expected, and we believe that their significance is practically the same. (See page 543.)

MICROBLASTS.—These cells have a nucleus the size of a normoblast, but the protoplasm is exceedingly scanty and often ragged on the margin. The nucleus is usually pycnotic. Whether these cells are derived from normoblasts as degeneration forms, or whether they are preformed and have the same significance as normoblasts, is not yet known. They occur in pernicious, also in severe secondary, especially the post-hemorrhagic, anæmias.

The presence of nucleated reds was noted in 57 of 69 of our cases. In 13, definite blood crises were present; that is, more than 50 nucleated reds per 1000 leucocytes. This is rather an arbitrary line, and yet we have found that, in our cases at least, it corresponded quite well with the blood pictures. In all cases normoblasts occurred, while in 40 (58 per cent.), megaloblasts also. In the other six cases normoblasts and intermediate forms occurred.

In these 57 cases there were 63 periods during which nucleated reds were present. Of these 63 periods, in 26—that is, in 41 per cent.—there followed a gain in the red blood-cells; in the rest, either no gain or a loss. Of 14 periods without nucleated reds, during 8 there was a distinct gain.

In 13 of our cases (19 per cent.) blood crises were present. Five of these cases died. Of the 50 or more nucleated cells per 1000 leucocytes constituting the crises, the normoblasts varied from 5 to 3128; the intermediates reached even 212, and the megaloblasts 44. There seem to be two definite forms of blood crises, those

in which normoblasts largely predominate and those in which the intermediate and megaloblasts are also present in considerable numbers.

It is the normoblastic crises particularly which are followed by a rise in the red count; those with many megaloblasts are less inefficient or occur in a condition of the bone-marrow when it cannot regenerate the blood. They appear especially when the patient is losing ground.

The most remarkable blood crisis lasted for nineteen weeks. During this time the red blood-cells, at the beginning 1,902,000, rose to 2,562,000, and then finally dropped at death to 1,328,000. During this time the leucocytes varied from 3000 to 5000 until the day of death, when there were 16,000. During the whole period the number of normoblasts per thousand of leucocytes was almost always above 500, reaching on one occasion 1164, a little later 1032, and finally 3128. On this day the leucocytes were 4600; hence the total number of normoblasts per cubic millimetre was 14,388, of intermediate forms 460, and megaloblasts 138 per cubic millimetre.

**Hæmoglobin.**—The hæmoglobin is much reduced, rarely above 50 per cent., and often as low as 10 per cent. The color-index is normal or high, a point of the greatest importance in determining the nature of the anæmia. In our cases on admission the hæmoglobin averaged 34 per cent., and the color-index in 80 per cent. of the cases was over 1, an average of 1.1, and in 2 cases as high as 1.9.

Ewing considers that the index is low in the chronic cases and high in the acute. For it to rise is considered a bad sign, indicating as it does a falling count; with improvement there is always a lowering of the index due to the newly formed cells, which are of lighter weight than normal.

The high color index has received various explanations. Some say it is the result of abnormal "globular richness," by which they mean that the individual cells contain an abnormally large amount of hæmoglobin, and this idea is confirmed by estimations made of the weight of the cells. Others say it is explained by the large number of macrocytes present, and the hæmoglobin curve does run fairly parallel to the curve of the number of these large cells. Others ascribe it, and with good reason in some cases, to blood counts which are incorrect since they do not include a great many of the microcytes which are so easily overlooked, and yet which contain no small amount of hæmoglobin. And, lastly, others say that in pernicious anæmia there is hæmoglobin free in the plasma. Many believe that the chemical composition of the red blood-cells is not normal; their nitrogen has been found increased (v. Jaksch), hence the name "hyperalbuminæmia rubra;" more iron is sometimes present than albumin to complete the hæmoglobin molecule, which means that iron is either increased in the hæmoglobin molecule, or is present in other combinations. Additional evidence for this is the hæmatogenous jaundice, and some have found iron compounds in the plasma, which may mean merely poor technic. Taylor considers the high color-index as an optical illusion; Capps, that color-index never surpasses volume index; Grawitz (and this appeals to us very strongly) warns against hæmoglobin determinations with an ordinary hæmoglobinometer and emphasizes the error of overlooking microcytes in blood-counting. He considers that the best test of the index is the appearance of the cells, and thinks that the inequality in the distribution of protoplasm and the production of poor cells are the prominent features of pernicious anæmia. Bengançon and Labbé think that from their appearance one does not get the idea that the cells are overrich in hæmoglobin. It is our opinion that they are. Many of the large cells in fresh specimens will be seen, when compared with normal ones, to be of a darker tint, but whether this tint is due to an

increased amount of hæmoglobin, to the greater optical thickness of a spherical cell, or to chemical changes of the hæmoglobin, it is difficult to say, and perhaps all elements enter. These large cells show little biconcavity, and often appear somewhat biconvex. That changes in the hæmoglobin can make the red blood-cells appear darker is illustrated by many degenerating cells, by cells picked up by phagocytes, and by the brassy cells of malaria.

It is most important to remember that those hæmoglobinometers with a color prism do not give accurate readings in the lower half of the scale unless they are standardized at various points along the prism, and an error of 5 per cent., so insignificant in normal blood, changes the index considerably when added to a total of 10 per cent.

The hæmoglobin during the course of our cases ran as a rule parallel with the red blood-cells. As the cases became worse the index slowly rose, and at death averaged 1.5. This may have been due to the tendency to form large cells.

**Leucocytes.**—In severe and uncomplicated cases there is always a leucopenia. Cabot's average was 3800, and in 72 of 110 cases below 5000. The leucocytes in our cases on admission averaged 4600. This includes all cases, even those with a leucocytosis due to complications. In 75 per cent. of the cases the count was under 5000. They may go as low as 1500 or 2000, and sometimes before death as low as 500 per cubic millimetre. Their number runs parallel to that of the red blood-cells, as a rule. A leucocytosis means either a complication, as pneumonia, some pus process, a blood crisis, in which case the large number of leucocytes may even suggest a leukæmia; and lastly, at death, the picture may be leukæmic (100,000).

Very roughly the leucocyte count ran in our cases parallel to the red count, and at death varied from 660 to 16,000, and averaged 5950.

Of our 81 cases, in 55 (70 per cent.) at some time during their stay the count fell below 3000; in 32 (40 per cent.), below 2000; in 9 (11 per cent.) it fell to 1000 or below. The very low counts, 1000 or below, are found only in the severest cases.

The percentage of *polymorphonuclear neutrophile cells* is roughly parallel to the total leucocyte count. This is best seen in the rise of these cells with the improvement of the case. Their percentage is lowest in the low counts, and the low count seems to be due to their diminution.

The percentage of the non-granular mononuclear cells varies inversely to that of the granular cells. The highest in our cases was 93 per cent. In the majority of the cases the percentage relation of the leucocytes tends to be constant whatever the total count of these cells, and indicates that these variations are due more to the distribution or dilution of the blood, perhaps stasis in the vessels, than to any real change in blood formula. On the other hand, there are considerable changes in total count, in which the absolute number of these mononuclear non-granular cells is quite constant.

Toward death the percentage of these cells rises, probably because the granular cells are formed in diminishing numbers. It is interesting

that the variations in the percentage of these cells show definite waves during the course of the disease.

The relatively high *lymphocyte* count is seldom a true lymphocytosis, but is due to an absolute decrease in the polymorphonuclear cells. The average of small mononuclears is 45 per cent. It may reach as high as 62 per cent. and before death even 79 per cent., and yet the absolute number be normal. This has been considered evidence that these lymphocytes arise in the lymph-glands and not in the bone-marrow. This decrease in the polymorphonuclear cells is the important feature, and in diagnosis excludes often cancer and septic anæmia, yet in the same case the count varies so much that it is not of very much importance.

In 12 (17 per cent.) isolated counts there was a true lymphocytosis. This was maintained in no case for more than one or two counts. Two of these were cases with a definite leucocytosis, while in the other cases the total count was not above normal limits. Hence a lymphocytosis may occur, but is not a common feature.

The *eosinophiles* averaged about 2.7 per cent. They may reach as high as 9 per cent., and are often absolutely increased. In extreme cases they may be diminished.

The *myelocytes* may reach 2 per cent. Their presence is more constant and their number greater than in any disease except leukæmia. In acute exacerbations of the disease they may reach even 29.4 per cent. of a total of 34,000 cells (Billings). Eosinophilic myelocytes sometimes occur, but rarely. In 23 of our cases myelocytes were present in numbers varying from 0.2 to 8 per cent. Nine of these cases were fatal. In 12 cases the percentage was not above 1 per cent. In 6 it was above 3 per cent.

In our cases the myelocytes occurred especially under two conditions; in cases with a very low count, even the lowest, in which case their percentage was the highest; for instance, of 1800 leucocytes, 8 per cent. were myelocytes. Again, they were much increased in cases with leucocytosis, as, for instance, a case with 14,400 leucocytes and 2 per cent. myelocytes; another with 11,600 and 0.5 per cent. myelocytes.

*Mastzellen* were present in 29 of our 69 cases. In 2 they were over 3 per cent., but in 8 over 1 per cent. Of the other 21 cases the average was 0.5 per cent. If these high percentages were really of *Mastzellen* it would show that in pernicious anæmia they show a definite increase hitherto not mentioned. We doubt very much that this is the case. In our cases of pernicious anæmia, in none were differential stains for these cells used, and in this disease it is quite common to find polymorphonuclear cells presumably of the neutrophile series without granules. So marked is this in some cases that it has been suspected that the body has lost its ability to form the neutrophile material (Ehrlich). This may



explain their high percentage. As is well known, in using Ehrlich's stain most non-granular polymorphonuclear cells are counted as Mastzellen. The high percentages occur always in cases with a low total count, the average of the above cases with over 1 per cent. being 3900.

Degenerated leucocytes are common; pale, swollen, and vacuolated, with the nuclei fibrillar. There is an increased number of neutrophile granules in the periphery of some cells. Hayem considers that they will imbibe a certain amount of hæmoglobin. Certain cases are reported in which the diagnosis between pernicious anæmia and acute leukæmia was said to be quite difficult; one, for instance (Williamson and Martin), in which the red blood-cells were 300,000, hæmoglobin 12 per cent., leucocytes 38,000, with the small mononuclears 99 per cent.; Westphal's case, with 816,000 reds and 24,000 leucocytes; Bezanson and Labbé's, with 550,000 reds, of which 3520 per cubic millimetre were nucleated, leucocytes 32,000, small mononuclears 66 per cent. (see page 614).

The absolute number of the eosinophiles is a splendid index of the course that the blood is taking, running in many cases parallel to that of the red blood-cells. During sixteen admissions there was a definite rise of these cells attending the improvement of the condition.

In several cases, with little change in the condition of the blood, the number of these cells was fairly constant, while in 10 cases these cells fell as the red blood-cells dropped, in 3 being absent at the time of death, and almost so in 2 others. These cells may drop as the patient goes down hill, even though the red blood-cells do not. Some cases are exceptions, as, for instance, in 8 there was no rise in eosinophiles as the blood improved; in 4 there was a rise, but without any accompanying improvement; while in one count, a terminal pneumonia, these cells were 220 in number at death. The explanation of these exceptions, however, is not difficult. In cases in which with apparent improvement there was no rise of eosinophiles it is to be noted that the increase in red cells was particularly rapid, averaging 43,000 per day. In 5 cases, with a considerable rise ending in a definite eosinophilia, the average gain of red cells per day (17,000) was slow, and lasted over a considerable period of time; while in those cases with a slight increase and not ending in a definite eosinophilia the rise in the red count was still more rapid, averaging 34,000 cells per day. This, we think, indicates that the slow rise of the red count over a considerable period of time is more surely due to new blood formation. The rapid changes in the red count may mean plasma changes, etc.

The number of eosinophile cells may not run parallel to that of the red cells, but their large numbers occur chiefly in those cases which are doing well or after they have already done well; that is, following



a rise of the reds these cells may be increased. They are present in particularly large numbers in those cases gaining very slowly.

A diminution in the absolute number of eosinophile cells may be of ill omen, as in one of our fatal cases. For fifteen days before death the red blood-cells remained constant; that is, the first of six counts was 2,832,000, the last 2,704,000, and the average in all was 2,700,000. The absolute number of eosinophiles at first was 183, shortly afterwards 180, and toward the end there were none at all.

**Platelets.**—The blood-platelets are decreased or even absent, often only one-twentieth the normal number. In other cases they are said to be increased (v. Limbeck and Sahli). Grawitz considers that they vary. Hayem found the count as low as 25,000 or even 15,000, per cubic millimetre.

**Coagulability** is usually decreased. The blood from a venesection does not separate into clot and serum.

**Serum.**—The changes in the plasma are important, since this constitutes 90 per cent. of the total blood. It loses very little of its albumin; for instance, there is a loss of 50 per cent. of the albumin of the total blood yet of the serum of only 8 per cent. This is very different, therefore, from the hydræmic anæmias after hemorrhage and those due to poor diet, in which the serum is most affected. It is an important diagnostic point also to exclude the anæmias of cancer and of sepsis (cryptogenetic infections).

The **specific gravity** averages about 1030, and may go as low as 1025.

The **solids** of the blood average but about 9 per cent. The water is increased to even 90 per cent. The greatest loss is in the albuminous bodies, which are reduced even to one-third the normal. This is especially due to the corpuscles, for the serum in the severe cases may be normal. In the plasma the serum globulin is alone decreased, serum albumin being practically normal.

**Chlorosis.**—This is a disease especially of young girls at puberty, the essential blood-feature of which is a reduction in the hæmoglobin. The count of red cells is almost normal; the cells show few signs of degeneration or destruction; the hæmoglobin formation is very defective, and there seems to be a polypasmia. It is the only good illustration of anæmia due to defective hæmogenesis, and differs from all other forms in the lack of evidence of blood destruction (v. Noorden), as shown by the poverty of the urine in pigment, the slight degeneration of the red blood-cells, and the absence of jaundice. Chlorosis is more a clinical than a blood picture, since this latter is well simulated by many secondary anæmias, for instance by the ordinary post-hemorrhagic form. We cannot make a diagnosis of chlorosis from the blood alone, since all secondary anæmias have some of its features.

Yet clinically the picture is sharp, so sharp that some will diagnosticate chlorosis without blood changes (Laache).

The blood features to be emphasized are: that in chlorosis there is a more uniform diminution in the size of the red cells, and a more uniform paleness, while in the secondary anæmias, even of a severe grade, the red cells vary widely in size, and a good many will be normal in size and color; in chlorosis the color-index is lower, a lymphocytosis more common, the nucleated reds more infrequent, and coagulation more rapid than in secondary anæmias.

The gross appearance of the drop is very pale, thin, and watery, and the blood clots rapidly.

The count of the RED BLOOD-CELLS need not be very much reduced, and yet in over 60 per cent. of the cases it is under 4,000,000 cells at the first visit (Reinert, v. Limbeck). Thayer's average of 63 cases at the first visit is 4,096,000; Cabot's, 4,112,000; Gräber's, 4,482,000, while Grawitz's cases varied from 3,400,000 to 4,300,000. The minimum count of Cabot's was 1,932,000; of Thayer's, 1,953,000, and of Hayem's, 937,360. Gräber, who claimed that in simple chlorosis there is no diminution in the count, which would indicate a complication, cites a maximum of 5,700,000 cells. Low counts are rare, and some complications may always be suspected, as ulcer of the stomach. The color of the red blood-cells indicates a marked diminution of hæmoglobin, their biconcavity is pronounced, the pessary form of cells common, and the cells stain very poorly (Plate I, 23, 24).

There is a quite uniform diminution in the size of the cells, and yet the large, pale, so-called "chlorotic cells" bring the average up to almost normal, the cells varying from 5.2 to 11.5 microns in diameter, with an average of 7.5. These large chlorotic cells are interesting, since many consider that they are dropsical cells,—that is, cells swollen because of the water they have imbibed from the plasma; and yet their number is usually few and the great majority of cells are almost uniform in size, and a little smaller than normal. There is not the large admixture of normal cells seen in secondary anæmia. Macrocytes are rare. Microcytes are more common. Schaumann and Willebrand say that at the height of the disease the small cells predominate, while during convalescence the large cells. Grawitz says the cells are largest when the case is at its worst. These large chlorotic cells may be very numerous, even one-third of all, at the height of the disease.

Poikilocytes and degenerated cells are rare except in the severer cases, and the polychromatophilia is considered by many to mean youth of cells, and, therefore, to be a sign of active regeneration (Grawitz). "The granular degeneration does not belong to the picture of chlorosis, but means some complication." Stengel and Pepper think it common.

Nucleated reds are very rare except in the severer cases, or during improvement, when blood crises may occur, although this is denied by some. They are much rarer than in the secondary anæmias. They are usually normoblasts, seldom megaloblasts.

**HÆMOGLOBIN.**—It is the reduction of the hæmoglobin which is the characteristic feature. This may be reduced to even 20 per cent. Cabot's average on first visit was 41.2 per cent.; Thayer's, 42.3 per cent. The color-index is, therefore, low, averaging 0.5, but in some cases it is as low as 0.3. Secondary anæmias never reach this level. The cause is the small amount of hæmoglobin in each cell and the large numbers of small cells. The volume of the red blood-cells is just about half the normal.

The average *leucocyte count* in Thayer's cases was 8467; in Cabot's, 7485; that is, the count is normal: a leucopenia is not uncommon. This is important in diagnosis, since in the secondary anæmias, especially those of cancer, there is usually slight leucocytosis. During the convalescence the leucocytes may increase more rapidly than the red cells, and there may be even a leucocytosis.

Grawitz and v. Linbeck say that the blood formula is normal. Most observers, however, even in mild cases, find the small mononuclears about 33 per cent. There is a slight absolute diminution in the neutrophile cells, and among them may be found cells approaching myelocytes, but typical myelocytes are very rare. The eosinophile cells are usually somewhat increased, averaging 3.5 per cent., and in some cases even 9.6 per cent.

During the last five years there have been admitted to our female wards but 13 cases diagnosed as chlorosis. Of these, but 2 were at puberty, and the rest from seventeen to twenty-five years old (relapses?). Of these, the lowest count was 2,600,000, the highest 4,000,000, the mean 3,700,000. Hæmoglobin, 26 to 49 per cent. Color-index, 0.36 to 0.63; mean, 0.47.

Leucocytes: lowest, 2400 and 3800; between 5000 and 7000, 6 cases; highest, 8000.

Differential counts made in 7 cases, all practically normal (even in the case with a total of 2400, there were: small mononuclears, 17.2 per cent.; large mononuclears and transitionals, 3.9 per cent.; polymorphonuclears, 77.1 per cent.; eosinophiles, 1.8 per cent.).

It is interesting that when they left the hospital all (9 cases thus examined) had gained practically the same, between 900,000 and 1,711,000 cells; mean, 1,100,000.

The reason why we now see so few typical cases of chlorosis is a mooted question, but we suspect it is the vast increase in the use of patent medicines which contain iron.

The **PLATELETS** are increased, as a rule; in fact, in no condition are they as numerous as in chlorosis. They also are large in size.

The *specific gravity* of the blood is low, sometimes reaching 1030. This is due to the loss of hæmoglobin, and in this disease alone does the

specific gravity run parallel to the hæmoglobin content. Grawitz states that it varies from 1035 to 1045; others put the figures at 1030 to 1050. Grawitz says that if it is under 1035 there is some complication.

The *alkalinity* of the blood is normal.

The *isotonicity* of the cells is low.

In the *serum* there is very little change, since there is no blood destruction, a feature which usually affects the plasma first. There is no hydræmia in chlorosis, yet the total plasma seems increased (polyplasmia). As the case improves, the number of the reds rises rapidly to normal; that is, "the anæmia is first cured" (Gräber), then more slowly are these light-weight cells replaced by those more normal in size and shape and in hæmoglobin content. Yet these variations in the count need not mean a new formation of cells alone, since the plasma changes must not be neglected; in fact, the first sign of improvement is an increase of specific gravity and an increased count due to the disappearance of some of the plasma, as shown by the polyuria and the disappearance of œdema. Later the signs of regeneration appear, also the gradual elimination of the faulty cells, the appearance of more normal ones, and the rise of leucocytes to even above normal.

**Leukæmia.**—Leukæmia is a disease marked by the constant presence in the blood of granular mononuclears or an increase of the non-granular cells with round nuclei, the immature cells of the blood-building organs which are not normally present in the peripheral blood. The blood formula is markedly changed. There is, as a rule, also a great increase in the total number of the leucocytes, and yet during the periods in which the count is normal the diagnosis can often be made from the large numbers of these abnormal cells present.

Leukæmia is rated among the primary anæmias, although the diminution in the red count is not an essential feature. The reason perhaps for the emphasis upon the anæmia is the old view that these white cells were immature red cells which had failed to develop hæmoglobin (Virchow). Interestingly enough, for certain cases of acute leukæmia there is now a reversion to this idea by some writers, the similarity in shape of the normoblasts and the lymphocytes, and the percentage relations both in the marrow and in the blood indicating that perhaps the mother cell of both is increased and produces the white cells chiefly.<sup>71</sup> Apart from this, the cachexia, which always arises sooner or later, is a very important feature of the disease.

Anatomically, it is a disease with lesions of the hæmatopoietic organs only. Formerly it was the pathological picture which was the most important, then the blood picture; as a fact, both are important, since the latter is not the disease, but a symptom of the former.

According to the blood picture three forms may be separated:

<sup>71</sup> See Reed, Amer. Jour. Med. Sci., October, 1902.

(1) Lymphatic leukæmia, "lymphæmia," in which the increase is of the non-granular cells.

(2) Splenomyelogenous leukæmia, "myelæmia," or "true leukæmia," with an absolute increase of all forms, but especially with the presence of the mononuclear granular cells.

(3) Mixed leukæmia, in which both the granular and non-granular mononuclear cells are increased.

Of all three forms may occur cases of acute leukæmia (see page 614).

Whether the disease is a disease of the lymph-glands on the one hand, and the bone-marrow on the other, or whether both non-granular and granular cells arise in the marrow alone, is a question for the physiologists to decide. From a hæmatological point of view it is to be emphasized that the abnormal cells are always young forms; that for many of the non-granular, mononuclear cells the name lymphocyte is a misnomer; that in the myelogenous and mixed leukæmias all forms of leucocytes are involved; and it is only in the so-called lymphatic leukæmia that one group alone is increased.

Leukæmia differs from leucocytosis not so much because of the higher white count as of the presence of large numbers of these unripe cells, and also of its chronicity. In the intermissions the diagnosis can often still be made from the differential count.

There is a tendency now to group all forms in one, and pathologically this may be justified, but not clinically, for there the line is generally quite sharp between the myelogenous and lymphatic forms, although some (Wolf especially)<sup>72</sup> claim to have demonstrated the transition from a lymphatic to a myelogenous type, and the reverse change seems to be even more common. In claiming such transitions. Wolff insists that cases in children must be excluded; a coexistent leucocytosis and lymphatic leukæmia must be thought of; and considers that one may recognize non-granular myelocytes. Grawitz sums up the question thus: that leukæmia is one disease, with various symptoms and various blood pictures, and with but one law, and that is its lawlessness.

**Splenomyelogenous Leukæmia (Plate I).**—In this disease there is a marked increase of all the granular cells, especially neutrophiles, also of eosinophiles and basophiles, and especially of the young forms of these cells with spherical or but slightly indented nuclei. The non-granular cells are also very much increased.

**Total Blood.**—In many cases of this form of leukæmia there is certainly an increase in the total volume of blood, as is shown before death by the dilatation of the veins, as well as at the autopsy table. Later on, however, in other cases, as death approaches, a diminution in the total blood volume seems to occur.

<sup>72</sup> Zeit. f. klin. Med., 1892, vol. xlv.

**Grossly**, the blood looks normal even when the leucocytes are almost equal in number to the red blood-cells. In extreme cases it has a pale, more opaque look, and flows sluggishly. Cases have been described in which the fresh drop resembled "chocolate mixed with cream." This must be very rare, as so many have never seen it. It is probably due to hæmoglobinæmia. When making smears the blood seems thick; hence it is hard to get good preparations (which appear granular); the diagnosis has often been made in this way. If the blood be allowed to settle and coagulate, there will form a grayish-white layer on the top of the clot, which may suggest the diagnosis. Coagulation is slow, and in the severe cases sometimes absent.

**Red Blood-Cells.**—As a rule these are diminished (Grawitz said always unless some factor concentrating the blood was present). In Taylor's cases there were none above 4,000,000. Very rarely is the count normal; very rarely is it much diminished, although in some cases it is. The cachexia, slight jaundice, increased urinary pigment, and the deposit of iron in the various organs show that a toxic hæmolysin is present. Cabot's average count was 3,120,000; Osler's, 2,850,000. In 9 recent cases with 11 admissions, the lowest count was 1,640,000; the highest, 3,800,000; mean, 2,800,000. As the leucocytes increase the reds decrease, and *vice versa*. There are exceptions, however, and the count may remain almost normal for a long time, some cases at 5,000,000. The anæmia may be due partly to the hemorrhages, which are so common, or to the albuminuria and the diarrhœa. The count may be almost as low as in pernicious anæmia, and attention is called to the fact that this oligocythæmia persists during those periods at which the leucocyte count is normal and the patient feels better. If the patient is seen for the first time at this period, the diagnosis of pernicious anæmia would certainly with justice be made (Taylor). The subjective condition of the patients depends little on the count of red cells, since they are ready to go home when this has changed but little.

**QUALITATIVE CHANGES.**—The anæmia is of the chlorotic variety, the cells being pale, with little hæmoglobin. This is best seen if stained with indulin or nigrosin. There is remarkably little degeneration, although the endoglobular areas do occur. Microcytes and macrocytes are rare; poikilocytes occur in all cases, but not many as a rule. The polychromatophilic degeneration and the basophilic granules are common, yet are never very numerous, and in some severe cases the reds are normal. Biermer's test was found positive in two cases.

**NUCLEATED REDS.**—These cells are remarkably numerous considering the mild grade of the anæmia; in fact, this is the condition *par excellence* in which to study them, as there is no disease in which normoblasts occur more constantly; yet their absence is not against

this diagnosis. Megaloblasts are found in many of the cases, and in some are quite numerous. The same is true of gigantoblasts, which may reach a diameter of 20 microns. This megaloblastic feature of the blood while marked in leukæmia is not so much so as in pernicious anæmia. Microblasts also occur. Karyokinetic figures in all stages of division are best studied here. Of Taylor's 16 cases, in 2 the number of nucleated reds varied from 60,000 to 70,000 per cubic millimetre, and one of the first effects of the arsenic was to reduce the number of these cells. Before death there may be but few, or there may be an increased number of these cells. It is of interest that marked rises in the white count are accompanied by rises in the number of nucleated red cells.

The hæmoglobin is reduced, the color-index being about 0.6. Osler's average of hæmoglobin was 42 per cent. In 9 recent cases the mean was 30; index, 0.54. The hæmoglobin is hard to estimate, since the leucocytes render the blood so opaque.

**Leucocytes.**—From the appearance of the fresh specimen the diagnosis may sometimes be made at a glance, not so much because of the large number of leucocytes as the large number of immature cells which are never present in normal blood. One may have a simple leucocytosis with the count as high as it is in some cases of leukæmia; in some cases of leukæmia the count is normal; and some post-febrile cases have a blood formula which for a time suggests leukæmia.

Counts of 500,000 are not rare. Cabot's average at the time of the first visit was 438,000; Osler's, 298,700. The counts vary, yet not as much during one admission as would be supposed, the daily counts maintaining approximately the same level for weeks, and during the same day we have not seen the great variations spoken of (*c.g.*, on one day the blood was counted each four hours: 146,000, 134,000, 141,900, 143,200). Some are cases with quite uniformly high counts—over 400,000; but the most have moderate counts,—from 100,000 to 300,000 (63 per cent. of 51 cases), while fewer are below 100,000. One case may belong at different times to each of these groups, but during a hospital admission it keeps in one group. There are periods when the count is normal, yet even then the differential count will usually give the diagnosis. (In three of Taylor's cases there were no qualitative changes.) In some other conditions neutrophile myelocytes occur, but not with increased eosinophiles and basophiles; mononuclear eosinophiles are rare in other conditions.

**DIFFERENTIAL COUNT.**—All the cells of normal marrow appear in the blood. Among these the *neutrophile myelocytes* in enormous numbers are the predominating cell. The very large myelocytes occur, with a large chromatin-poor nucleus which stains palely, is hard to make out, and is often in an eccentric position; these cells are seen only



in this and in some diseases of children; they are sometimes even 30 microns in diameter (Cornil's myelocytes). Also smaller myelocytes, about the size of an ordinary leucocyte and with a centrally placed round nucleus which stains well, are seen. Lastly, dwarf myelocytes about the size of red cells occur. All transitions between these largest and smallest myelocytes may be found. Mitoses are more or less common. The number of granules in these cells varies considerably, some being full, in others there are a few, and still other cells are confusing, since different persons will not agree as to whether they are granular or not. Grawitz emphasizes the large non-granular cells, some of which are very large, with a homogeneous body and a large pale nucleus which is often seen without protoplasm, and which break up rapidly; others are medium sized, with basophilic protoplasm staining intensely, and a medium-sized nucleus; in other similar cells beginning granulation can be seen. These are the transitional forms of myelocytes. There are also myelocytes about the size of a leucocyte, with rather small compact nuclei. This is the form seen in inflammatory leucocytosis.

*Eosinophile myelocytes* are found, sometimes in large numbers, but they are never as numerous as the above. There also occur all transitional forms between these and eosinophile leucocytes.

*Polymorphonuclear Neutrophiles*.—While these are relatively diminished (Cabot's average 46 per cent.), their absolute increase is considerable, even to about 50,000. Anomalous cells are common, some very large, even 20 microns in diameter, some small, or dwarf cells, 4 microns in diameter, a variation in size which never exists in leucocytosis. Again, cells with unusually shaped nuclei occur, and cells with more than one form of granule. The granules may vary in tint, which depends partly on the method of fixing; in one case all cells were described as non-granular. Free granules from the many cells which have broken down can be found in the plasma.

*Lymphocytes*.—The percentage of these cells is reduced, the average being 10.6 per cent., but usually there is an absolute increase. These cells vary much in size, and among them are some which it is very difficult to tell from myelocytes. One finds also the large mononuclear cells which are common enough in the marrow, but which never reach the blood normally or in other diseases. Some have very irregular shapes, some a few granules.

The *large lymphocytes* have a scanty ragged protoplasm and a large chromatin-poor nucleus. These are Fränkel's unripe cells, supposed to be characteristic of acute leukæmia, but occurring also in the chronic types. *Large mononuclears*, both those of the normal blood and those mistaken for myelocytes, occur in large numbers. Of the latter the nucleus is often very basophile, the protoplasm is finely fibrillar, and



distinctly basophilic or acidophilic. These cells before the Ehrlich's stain was used were reckoned as myelocytes.

*Large phagocytes* (splenic cells?) are sometimes present, numbering in one case 1.2 per cent. of the leucocytes (total count 216,000).

*Eosinophiles*.—In this disease there is usually an absolute increase of these cells. Ehrlich, indeed, stated that he would not make the diagnosis of leukæmia unless an absolute count of more than 250 cells was present. Since then three cases at least have been reported<sup>73</sup> of undoubted leukæmia, but at times without a single eosinophile cell, and others with extreme fluctuations in their numbers. As a rule the minimal number in leukæmia is about 3000, the average percentage 5.1, and the average absolute number 11,000. These cells occur in all modifications like their neutrophile analogues, the large myelocytes (formerly said to be the characteristic cell), the medium-sized ones, the dwarfs, and the ordinary leucocytes. The eosinophile myelocytes may in this disease form the majority of the eosinophile cells.

*Basophiles*.—Ehrlich considered that there was always an absolute increase of the Mastzellen in leukæmia, and that this was the only condition in which they were increased. Their absolute increase may be above that of the eosinophiles, and is always proportionally higher. In one of Lazarus's cases they reached 47 per cent.; in one of Cabot's, 10 per cent., while Taylor mentions a case with an absolute count of basophiles of 140,000. Taylor also states that in two cases no Mastzellen were present.

CHARCOT-LEYDEN crystals may be found in the blood after it has stood for a while, but also in the fresh blood, as was shown by splenic puncture. Some observers, however, including v. Limbeck and v. Jaksch, have never found them. They are normal in the bone-marrow and are present wherever the eosinophile cells are increased. Bezançon and Labbé say that leucin spherules also will separate spontaneously.

Ehrlich considered that from the examination of the smear alone the diagnosis of this form of leukæmia could be made. The six points which he emphasized were: the presence of neutrophile myelocytes; eosinophile myelocytes; an absolute increase of eosinophiles and of Mastzellen; the presence of atypical cells, among which are dwarf eosinophiles and neutrophiles, both mononuclear and polynuclear; cells in mitosis; and, lastly, the large number of nucleated reds. As mentioned above, however, the absolute number of eosinophile cells is no necessary part of the blood picture. And, indeed, any one of these points may fail for a while, at least.

A great many of the leucocytes show signs of extreme degeneration. Ewing considers that eosinophile myelocytes with granules of

<sup>73</sup> See Simon, Am. Jour. Med. Sci., No. 125, 1903.

very unequal size and density of stain are pathognomonic of myelocythæmia. In some cases the protoplasm is swollen, hyaline, or vacuolated. Nuclei surrounded by granules scattered widely through the plasma, the protoplasm evidently having disappeared, are very common pictures. In the nuclei, karyolysis, vacuolation and karyorrhexis are common; pycnosis perhaps less so. Degenerated leucocytes are always present in leukæmia.

In the diagnosis the point upon which emphasis was formerly laid to distinguish it from an extreme leucocytosis was the large number of white cells. This distinction does not in the least hold, since there are periods of leukæmia with a count normal or even subnormal, and yet during this time the formula may be that of leukæmia. The presence of myelocytes alone does not give the diagnosis, since in cases of extreme leucocytosis a few myelocytes will usually be present. These myelocytes, however, are usually about the size of the ordinary leucocyte, and are never the very large cells which occur in leukæmia; also eosinophiles and Mastzellen are not increased. The diagnosis is especially difficult in children. In some cases autopsy alone will decide it.

A recent case on first admission had 443,000 leucocytes; was readmitted in fourteen months with a count of 9700; the count remained low till his discharge twenty days later with 100,000. On the day with the lowest count, 6000, the differential was: s. m., 3.8 per cent.; l. m. and tr., 3.6 per cent.; pm. n., 70.8 per cent.; eos., 3.8 per cent.; neutroph. myeloc., 8 per cent.; Mastzellen, 7.6 per cent.; normoblasts, 23; intermediates, 15; megaloblasts, 5 (per 1000 leucocytes). Hence even at this time a diagnosis could have been made.

Variations in the count are extreme over long periods of time; the daily variations are sometimes considerable, as in one case reported, with 122,500 at ten A.M., at four P.M. on the same day the count was 235,000.

With improvement in the condition the count may drop almost to normal. At this time the formula should help in the diagnosis, and yet this is not always true, since in some cases the characteristic blood picture has entirely disappeared. During such a period it would be impossible to differentiate a case with low red count from one of pernicious anæmia by the blood alone; and, indeed, there are cases reported of a transformation to pernicious anæmia and *vice versa*. Following the long-continued use of arsenic the count drops in a remarkable way, to rise at once after the drug is discontinued. Türk mentions a case<sup>74</sup> in which the leucocytes ranged from 258,000 to 370,000. After arsenic treatment they fell to 3000 to 6000 (0.5 per cent. myelocytes, 6.6 per cent. Mastzellen). It is a question how much improvement this indicates, as it may be an "exhaustion" of the bone-

<sup>74</sup> Deut. med. Wochenschr., 1904, No. 50.

marrow. Following X-ray treatment remarkable drops have been reported, even from 693,000 to 6300; the leukæmic character, however, was never lost. The red cells rose to nearly double in this case (Joachin and Kurpjuweit).

The infectious diseases which are survived, and this is rare, have a remarkable effect not only on the blood picture but also upon the blood-forming organs. This is particularly true of typhoid fever, influenza, miliary tuberculosis, *et al.* Chronic tuberculosis has very little influence. In Dock's case,<sup>75</sup> due to grip, the cells fell from 367,000 to 5000, then in six weeks returned to 157,000, and in one year to 461,000. The fall is sometimes extreme, as from 40,000 to 470. Some cases preserve the leukæmic formula, others do not. During the acute infection, when the count falls almost to normal, there is also a remarkable reduction in the size of the blood-building organs, and both sometimes return to their former condition in a few days after the infection is past; but not always, as in a case reported by McCrae; and others are on record in which at autopsy all signs of leukæmia had disappeared from the bone-marrow. In other cases, however, there is a rise instead of a fall, as in Müller's case of sepsis the leucocytes dropped from 246,900 to 57,300, then rose; in v. Limbeck's case of pneumonia they fell from 140,000 to 43,500, and then, as the other lung became involved, rose to 172,000. As the count drops the percentage of polymorphonuclears rises, the picture thus approaching that of a leucocytosis.

Late in the disease there may be a marked predominance of the large non-granular leucocytes, and there is good reason for the opinion that some of these are myelocytes without granulation, as if the body had lost its power to form the neutrophile material (Ehrlich).

**Platelets.**—In this form of leukæmia the platelets are markedly increased, even reach their maximum.

The *water content* is increased to 81 to 88 per cent.

The *specific gravity* is low, even 1036; that of the plasma about normal.

The *alkalinity* is somewhat decreased by the organic acids formed from the breaking down of leucocytes. Formic, acetic, lactic, and succinic acids have been found in the plasma. The xanthin bodies of the plasma are increased. Deutero-albumoses have been found. These are not present in lymphatic leukæmia, and are supposed to be digestive products of the leucocytes from a ferment provided by the polymorphonuclear cells. Taylor says that the nitrogen of the leucocytes is almost double. Nucleo-albumin is found in the serum, and uric acid, 22.6 mg. per 100 cc. Coagulation is increased, in one case so much so that the red blood-cells could not be counted with a pipette.

**Lymphatic Leukæmia (Lymphæmia)** (Plate II, A, B, C).—In this form of leukæmia is a marked increase of the mononuclear non-

<sup>75</sup> Am. Jour. Med. Sci., 1904, vol. cxxvii., a very exhaustive study of this subject with a review of 50 cases.

granular cells. Despite the name, these cells are not all lymphocytes even in morphology, to say nothing of origin, but are mononuclear, non-granular cells of many sizes and forms. As a rule, while a variety of such cells are present, there is usually a predominance of one particular size; in some cases, the small mononuclear cells with a very narrow ragged rim of protoplasm; in others the cells are all of the large lymphatic type; in other cases the majority of the cells resemble the transparents, and in still others the transitionals of Uskow. In some cases the protoplasm of these large cells is basophilic; in other cases it is distinctly acidophilic; sometimes enormous cells are found.

As a rule there is proliferation of the lymphatic tissue of the body, and yet this is not always evident, as is seen in some cases in which there are no palpable lymph-glands. In some of these cases there may be considerable proliferation of the large masses along the intestines; in still other cases the lesion seems limited to the bone-marrow. Some interesting cases begin with a great enlargement of the lymph-glands, the blood picture appearing later and coincident with it a diminution in size of these glands. This was true of a patient who in September showed normal blood and a general enlargement of the lymph-glands. The following January he was readmitted with a leucocyte count of 110,000, chiefly the small cell variety. It is possible that the leukaemia does not begin till the disease of the lymph-glands has reached the marrow (Pappenheim).

**Red Blood-Cells.**—There is a much greater anaemia in this form than in the splenomyelogenous, and yet here also the count may remain normal for some time; later, however, cachexia with anaemia begins. In two of the chronic cases the count remained above 4,000,000 during a long stay in the hospital and until death. A remarkable case is reported (verbally) by Dr. Hazen, of eighteen months' duration, with reds 960,000, leucocytes 250,000, nearly all of the small lymphocyte variety. Cabot's average on first admission was 2,730,000; Osler's, 2,294,000; the average given by Hirz and Labbé is 1,829,000; by Petit and Weil, 1,292,000. The greatest reduction occurs in cases which show at autopsy the bone-marrow most involved, and in the acute cases, and the more acute the case the less the glandular enlargement. Nucleated reds are rare, sometimes absent, yet in the very severe cases there may be as many as in the splenomyelogenous variety. In one of our cases, with a total white count of 12,000, there were 150 normoblasts, 169 intermediates, and 20 megaloblasts per 1000 leucocytes,—a typical megaloblastic crisis. V. Limbeck describes them as astonishingly scarce.

The red counts remain very constant, often despite great fluctuations in the white cells (*e.g.*, 2,640,000 red cells, 105,000 leucocytes; in nine days 2,750,000

reds and 328,800; two weeks later, 2,892,000 reds, 410,000 whites; and again in two days, 2,928,000 and 480,000 whites).

In a case with pleural and ascitic fluids (chylous) repeatedly tapped, with profuse diarrhoea, and finally death from streptococcus septicæmia, the white cells showed very slight variations. They were 133,400 on admission, rose to 242,000, and at death numbered 133,000; the red cells were often above 5,000,000. They were 4,912,000 on admission and 5,340,000 at death.

The hæmoglobin is diminished, Osler's average being 37 per cent.

The leucocytes are increased, on an average to 144,800 (Osler) or 141,000 (Cabot). In this form also there may be aleukæmic periods, which may last even six months. Just before death the count usually rises. One of our cases during the last ten weeks before death had a leucopenia of even 1900 cells. The count may be as high as in the myelogenous form, but this is rare.

**DIFFERENTIAL COUNT.**—Grawitz classifies the cases as, those in which the small mononuclears predominate; those with a predominance of medium-sized cells with basophilic homogeneous protoplasm; and those in which the cells which predominate are very large and are for the most part degenerated. Yet all these forms may occur together in varying percentage in the same case, and vary at different stages. Roser thinks that in cases in which the lymph-glands are particularly involved it is the smaller cells which are increased, e.g., 99 per cent. of 117,000; and in those in which the lesion is particularly of the bone-marrow, the larger cells. Grawitz mentions an increase of the larger cells as simultaneous with a decrease in size of the lymph-gland. These mononuclear cells are often 90 per cent. of the total number, even 98 per cent., and in one of Osler's cases 99 per cent. A marked feature of the blood picture is the degeneration of these leucocytes. During the course even 10 per cent. and just before death even 75 per cent. may show some sign of degeneration, either of the protoplasm, or pycnosis and fragmentation of the nucleus. It is interesting that so few cells in the blood show mitotic figures, while in the bone-marrow the proliferation is very active. Many lymphocytes are very small, often even smaller than red blood-cells. They stain faintly in Ehrlich's stain, deeply in methylene blue; the protoplasm is scarcely seen, or is ragged and degenerated, the nuclei are round or indented, and even fragmented, with a sharp margin, and contain clear areas. Wolff thinks we should separate lymphatic from lymphoid leukæmia, the former being of lymph-gland origin, the latter myelogenous.

*Polymorphonuclears* are rare. *Eosinophile* cells are noticeably absent. In a pure case *myelocytes* are not present, although it is hardly wise to call the case mixed leukæmia if one be found. *Mastzellen* are absent, as a rule. In this form of leukæmia an acute infection may cause a drop of the total count or a true leucocytosis. Autopsy has shown the leukæmia cured. As a rule, even when the

count is low, the mononuclear cells will be 90 per cent. Wende's case <sup>76</sup> is a good illustration. The result of a streptococcus infection was a drop from 45,000 to 1600 leucocytes, but of small mononuclears from 95.3 per cent. to 88 per cent. The result of an acute infection is sometimes the appearance of a few myelocytes. Other cases show a marked increase in the count, as in Müller's case of chronic septicæmia it rose from 180,000 to 400,000.

In one case in this clinic the man was admitted with double tertian malaria and a lymphatic leukæmia of 105,000 (small monos., 83.6 per cent.; large monos. and tr., 7 per cent.; pm. n., 5.8 per cent.; eosinoph., 0.2 per cent.). One week after the malaria was cured the count rose to 328,000 and two weeks later to 480,000, with 97.2 per cent. small mononuclears. At this time there were 3 normoblasts, 3 intermediates, and 4 megaloblasts per 1000 leucocytes.

V. Limbeck considers that the blood picture alone is not enough for a diagnosis, since in some cases of sarcoma the blood presents a similar picture.

**Acute Leukæmia.**—This form of leukæmia, which has of late attracted a great deal of attention, is characterized by the brief course,—from six days to nine weeks (*leukæmia acuta et acutissima*),—the severity of the symptoms, the frequency of the hemorrhagic diathesis, the rapidly developing cachexia, and death. It occurs chiefly in young persons. The great majority of the cases are of the lymphatic type, but a few of the myelogenous variety have recently been reported; other cases are best described as mixed. The cases of the acute myelogenous form are collected by Gardinier,<sup>77</sup> who reports one and reviews eleven others, and also two doubtful cases (see also Billings and Capp).<sup>78</sup>

In all cases the anæmia is extreme, even below 1,000,000 red cells, and in Arneth's case 256,000 reds and 10 per cent. hæmoglobin.

The cases of acute lymphatic leukæmia are well reviewed by Rosenburger,<sup>79</sup> and acute leukæmia in children especially by Churchill,<sup>80</sup> who reports one case and reviews 28 others. The disease occurs even in the new-born child. The lowest red count was 750,000, after a severe hemorrhage. The leucocytes varied from 6000 to 810,000 (in a twenty-month-old child). The lowest counts came always just before death, which a falling count portends. Of these 29 acute cases in children, 25 were lymphatic (2 of the small-celled type, 3 large, 1 mixed); myelogenous, 2 mixed, 1 uncertain. Churchill's case had 99 per cent. small mononuclears, and many of them degenerated. The

<sup>76</sup> Am. Jour. Med. Sci., vol. cxxii., 1901.

<sup>77</sup> Johns Hopkins Hosp. Bull., October, 1904.

<sup>78</sup> Am. Jour. Med. Sci., 1903.

<sup>79</sup> Ibid., 1904, vol. cxxviii. p. 583.

<sup>80</sup> Ibid., 1904, vol. cxxviii.

anæmia is profound. It is of interest that the more acute the case the less the enlargement of lymph-glands and spleen. A good illustration of this type is Pfannkuch's case, which ended fatally in three days. The reds numbered 2,500,000, and the leucocytes, 1,000,000 (s. monos., 76.5 per cent.; neutrophilic myelocytes, 10.6 per cent.; neutrophile leucocytes, 12.2 per cent.).

Türk's case is a good illustration of the myelogenous form; red cells, 1,060,000; hæmoglobin, 19 per cent.; leucocytes, 42,000 (s. monos., 14 per cent. pmn. n., 32 per cent.; myelocytes, 47 per cent.). Certain blood conditions resemble acute myelogenous leukæmia. These are: an acute exacerbation of a chronic myelogenous leukæmia; a lymphatic leukæmia complicated by an acute infection; an acute lymphatic leukæmia of the large-celled variety (since it is not easy to distinguish these cells from myelocytes); an acute infection causing grave anæmia, in which case even 14 per cent. of the leucocytes may be myelocytes; an acute exacerbation of pernicious anæmia; and, finally, malignant disease of bone-marrow. A large number of nucleated reds would suggest this last condition (Billings and Capps).

In many cases the clinical picture is that of an acute infection, and very likely such cases will in time not be considered as leukæmia. Clinically it is the anæmia that attracts attention, and the relation between these cases and pernicious anæmia is very interesting.

There is no type of cell characteristic of this form; Fränkel's unripe cell occurs commonly, but by no means exclusively here; in 3 of McCrae's 5 cases the small lymphocytes predominated. The cells are much more uniform than in the chronic forms, yet some are cases with large cells, some with medium, and some with small. In some instances nearly all the cells have a basophile protoplasm; in others it is acidophilic (Plate II, C).

The *red cells* show few changes; as a rule nucleated reds are scarce, yet in Herrick's case they numbered 1,800 per cubic millimetre, of which some were megaloblasts (but see McCrae's case). The drop in count showing rapid blood destruction is a striking feature.

But in some cases the leucocyte count is not high or even above normal; then for diagnosis the lymphocytes must be always high (Klein). Such cases at first resemble pernicious anæmia.

Auer (Am. J. Med. Sci., June, 1906) described very interesting reds in the cytoplasm of the large mononuclear leucocytes of a case of acute leukæmia.

The cases of acute leukæmia in this clinic have been reported by McCrae.<sup>51</sup> They numbered five, all of the lymphatic type. Their average duration was six weeks, varying from twelve days to eight weeks.

On admission, average of hæmoglobin, 35.4 per cent.: of reds, 1,822,000; of

<sup>51</sup> Brit. Med. Jour., February 25, 1905.



leucocytes, 104,000; highest, 326,000 (hæmoglobin, 45 per cent.; reds, 3,000,000); lowest, 57,800 (reds, 748,000; hæmoglobin, 16 per cent.). In one case, in fifteen days the reds fell from 3,000,000 to 1,450,000. Color-index is high, 0.93 to 1.4. The small mononuclears varied from 94.2 to 99.4 per cent., and in three the small lymphocytes were the prevailing cell, unusual in this form of leukæmia. In one case occurred many large lymphocytes which were actively motile. Nucleated reds were absent in two cases, 2 per 1000 leucocytes were found in three cases and in the fifth case 310 per 1000 leucocytes; *i.e.*, 3720 per cubic millimetre, of which 7 per cent. were megaloblasts and 48 per cent. intermediates. McCrae emphasizes the high color-index of these and of the cases in literature, and finds that of 45 cases in 24 the red count was below 1,500,000, and in 38 of the 45 below 2,500,000. Of 40 cases from literature, in 20 the color-index was 1 or above, and in 20, under 1. The low count and high index of the primary anæmias seem to him a special feature of this type of leukæmia. A most remarkable case was recently admitted with reds, 752,000; hæmoglobin, 17 per cent.; leucocytes, 880,000, large-cell type.

Of 13 cases of acute leukæmia in children, collected by McCrae,<sup>82</sup> the anæmia was severe (highest red count, 2,350,000; lowest, 1,000,000), color-index high; the red cells were of normal appearance; no nucleated reds were found in 7 cases, few in 4, and megaloblasts in but one.

The leucocytes varied from 21,000 to 209,000; there was no relation between acuteness and character of cells. The absolute number of polymorphonuclears was about normal in all cases. In these cases the anæmia is the important feature; the leukæmia would not have been suspected without blood examination.

**Mixed Leukæmia.**—These cases may best be described as a lymphatic leukæmia with a considerable number of myelocytes, both eosinophile and neutrophile. A few myelocytes may occur in lymphatic leukæmia, and the question is one of drawing a line.

The cause of leukæmia is still to be discovered. A few words, however, may be said concerning Löwit's organism. In the splenomyelogenous form he described

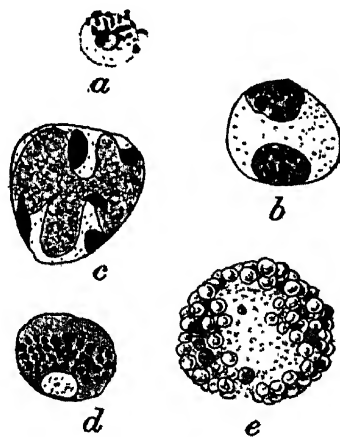


FIG. 115.—*a, b, c, d*, four leucocytes containing Löwit's organisms (copied from Löwit); *e*, large granular (and vacuolated?) cell of bone-marrow.

*Hæmamœba leukæmiæ magna*; specific bodies occurring in the large and small mononuclears, both non-granular and granular, rarely in the polymorphonuclear cells, never in the reds, sometimes free in the plasma. Their size varies much, and sometimes all varieties in the same cell. This he considers is due to multiple infection, while some of the groups of small cells he considers evidence of multiplication. They are in or on the leucocytes, often in vacuoles or splits of the

<sup>82</sup> Johns Hopkins Hosp. Bull., May, 1900.



protoplasm. They look as if they were amœboid when they died. They can be stained by a particular method, but they differ much in their staining qualities. Navicular bodies or crescent bodies are found which first suggested to Löwit the parasitic nature of the disease, since they resembled similar bodies in the coccidia and the hæmosporidia. These were never found in other conditions. He considers that he can exclude artefacts. He found them in the blood-building organs, and states that he got positive results from rabbit inoculation.

In the lymphatic form he describes *Hæmamœba leukæmiæ parva*. This he found, however, in but one of five cases studied. They are smaller than the preceding, seem more amœboid, are found especially in the blood-building organs, very rarely in the peripheral blood, points of resemblance analogous to the æstivo-autumnal malaria parasite. Those forms which he considers segmenters have a different arrangement and fewer segments. In this form no resting stage was found, and he is not certain of navicular bodies.

These parasites of Löwit have been the subject of a very animated discussion, particularly between himself and Türk who considers that they are much altered basophile granules or artefacts. The technic to demonstrate them is difficult and his findings unsatisfactory. We would expect the protozoa, if such they be, to show, as do others of a somewhat similar class, a little more uniformity in size, and a life history analogous to theirs. Studying Löwit's plates it would seem difficult to bring order out of such a chaos of forms. By unanimous consent the question has been allowed to drop, and yet in the blood specimens we have seen stained with his method even if all were artefacts they were beautiful pseudoparasites.

**Pseudoleukæmia.**—Under this heading have been grouped a great variety of diseases, including Hodgkin's disease, which now, by removing a superficial gland, can be easily excluded; tuberculosis of the lymph-glands, which can be recognized by the tuberculin test; lymphosarcoma and malignant lymphomata, which the pathologists say can be recognized anatomically. Others include splenic anæmia. Hence scarcely anything is left in the group of pseudoleukæmia, and it is the opinion of some that a "true" pseudoleukæmia is still to be proved (Reed); that is, a disease with the anatomical features of leukæmia, but not its blood-changes.

By the term pseudoleukæmia is generally meant a condition the clinical picture of which (the swollen lymph-glands and the cachexia) is that of lymphatic leukæmia, but without its blood-changes, and such cases are common enough, as the above list shows. Some cases which could be brought under this head are certainly early stages of lymphatic leukæmia, or cases during aleukæmic periods, yet other cases are chronic, even for eight or ten years.

**Hodgkin's Disease.**—The blood features of this disease are those of cachexia. In the eight cases reported by Reed the red count varied from 3,232,000 to 5,264,000 on admission. One case was as low as 2,670,000, but afterwards improved. These cases were, therefore, somewhat anæmic, and two showed a severe anæmia of the secondary type. At the onset the count may be practically normal and for months remain so despite the rapid growth of the lymph-glands. Then begin the changes of an ordinary secondary anæmia, often extreme, with at the end a count as low as 1,522,000, with degeneration of the red cells,

nucleated reds, and poikilocytes which are noticeably rare except at the late stages. The leucocytes are slightly increased, averaging about 12,000. In two of the eight cases there was an absolute increase of small mononuclears, but the lymphocytosis of Pinkus is by no means constant. The maximum count of small mononuclears was 38.6 per cent., the absolute, therefore, 5304, and the next highest was 4600 (36.8 per cent.). In two cases the small mononuclears were low, in one 2 per cent., or 310 cells, in another 9.4 per cent., or 940 cells. Grawitz considers that while the differential count is no aid in diagnosis, it is for prognosis; that a slight increase accompanies improvement, and a decrease, the reverse.

**Tuberculosis of the Lymph-Glands.**—In some cases of general glandular tuberculosis there is a normal red and leucocyte count, in other cases cachexia with a secondary anæmia. Some very interesting cases have a very low leucocyte count. In one case it was only 300 cells per cubic millimetre (Futcher). Most consider the blood of little value in the diagnosis of this condition.

Of this disease we have recently had 12 cases. Four of these showed a leucocytosis of from 11,000 to 29,000. The red cells were slightly decreased (two cases, 3,600,000 and 3,700,000, and 6 cases from 4,000,000 to 5,000,000), the hæmoglobin was much reduced and in 6 cases the index below 0.6. As a rule, there is no leucocytosis until a secondary infection occurs.

A case like the following is a puzzle for diagnosis. The woman, aged fifty years, had a red count of 4,000,000; hæmoglobin, 50 per cent.; leucocytes, 8000. She had tuberculosis of the lungs, also swollen lymph-glands, two of which were removed with an interval of one year, and both pronounced tuberculous. She had night-sweats and lost weight. Three months after the above blood-count the glands began to swell enormously; red cells, 3,000,000; leucocytes, 80,000, 96 per cent. of which were polymorphonuclear neutrophils. A little later the count had risen to 120,000; lymph-glands and spleen enormous. She received X-ray treatment, and in three weeks the leucocytes were 16,000 and the reds 2,100,000. She died soon after.

**Leukanæmia** is the name given by v. Leube to a group of cases formerly grouped by some with the acute leukæmias, and by others with the pernicious anæmias; the features of both are present. Rather than as an independent blood disease it is considered<sup>83</sup> a symptom of a large number of conditions,—injuries, hemorrhage, intoxications, infections, malaria, malignant growths, etc. There is severe anæmia with nucleated reds of all varieties, and a normal or increased white count, with many myelocytes, but no eosinophiles. Other cases resemble lymphatic leukæmia.

The anæmia usually precedes the increase in white cells.

#### BLOOD IN ACUTE DISEASES

**Malaria.**—The anæmia of malaria is important in diagnosis, since it is one of the earliest symptoms, the count dropping from 5,000,000 to

<sup>83</sup> Luce, Deut. Archiv. f. klin. Med., 1900, vol. lxxvii. p. 215.

even 500,000 in a few days. It is due both to the direct destruction of the corpuscles by the intracellular parasites and to the destruction of red blood-cells not containing parasites, the latter seen especially in those cases with hæmoglobinuria, in which the hæmolysis is serious.

The red blood-cells decrease after each paroxysm, slightly in the tertian and the quartan malaria, more in the æstivo-autumnal in which case there may be a drop of 1,000,000 cells after one paroxysm.

In 54 cases of æstivo-autumnal malaria the red cells were between 1,000,000 and 2,000,000 in two cases, 2,000,000 and 3,000,000 in 12, 3,000,000 and 4,000,000 in 20, and 4,000,000 and 5,000,000 in 12, above 5,000,000 in 8. In 56 cases of tertian the figures for these same limits were 1, 10, 28, 13, and 4 respectively. It is seen that in our cases these two forms differ little. The mean count for each was 3,500,000. But in this climate we seldom see truly pernicious cases.

In the "pernicious" cases the count drops somewhat also between the paroxysms. In Grassi's case there was a loss of 4,000,000 cells in six days. In one case the count at the end of thirty days was 500,000. Dionisi reports a case in which each chill cost from a half to one million red blood-cells. The greatest fall is in the earliest paroxysms, later less, until finally the count remains almost stationary despite repeated paroxysms. In cases with pernicious malaria and hæmoglobinæmia the anæmia is grave, with poikilocytes, endoglobular degenerations, occasional shadows, fairly numerous nucleated reds, increased platelets, and leucocytosis.

The regeneration of the cells in the tertian and quartan is rapid, the count often being restored before the next paroxysm, and anæmia occurring only after a long series of chills. In the æstivo-autumnal the recovery is slower, the new cells pale, varying in size and shape; nucleated reds are common, and since the anæmia is chronic the regeneration is slow, and grave anæmia may result. This slowness in regeneration is due also to the extensive necrosis and the resulting fibroid induration of the bone-marrow, which may be the chief seat of the infection, and to the accumulation of pigment in this tissue. The hæmoglobin suffers even more than the red blood-cells and returns to normal more slowly.

The leucocytes are almost always subnormal in malaria, an important point in diagnosis, except in the grave pernicious paroxysms. They rise slightly (to 6700, some say to a true leucocytosis) just before the paroxysm, and then steadily fall, reaching a minimum (average 2300) at the time the temperature is subnormal, and sometimes to as low as 1000 to 2000 cells.

In 82 recent cases of æstivo-autumnal malaria the leucocyte counts were, from 1000 to 2000, 3; 2000 to 5000, 8; 3000 to 4000, 21; 4000 to 5000, 15; 5000 to 6000, 14; 6000 to 7000, 8; 7000 to 8000, 4; 8000 to 9000, 2; 9000 to 10,000, 2; above 10,000, 5, one of these a pernicious case (14,500); mean 3500.

In 70 cases of tertian malaria the figures for these same limits were 2, 5, 11, 18, 10, 10, 5, 2, and 2; above 10,000, 5; highest, 16,500; mean, 4500.

The differential count shows a relative decrease of the polymorphonuclear neutrophiles, and an absolute increase of the large mononuclears. The mean averages found by Thayer are: Small mononuclears, 16.9; large mononuclears, 16.9; polymorphonuclear neutrophiles, 65; eosinophiles, 0.9 per cent.; in grave cases, myelocytes 2 to 3 per cent. (Cabot). The increase of the large mononuclears, very pronounced in the apyretic periods, is usually absent in the pyretic periods. If as the temperature falls these cells do not increase, it is evidence against malaria, while they are most valuable in the diagnosis of cases admitted after they have taken quinine, and hence without parasites in the peripheral blood. In the tropics Stevens and Christophers say that over 15 per cent. large mononuclears means an actual or a recent malaria; with 20 per cent. one almost always finds the parasite. This increase affects the cells which Uskow calls "transparent," a group of cells which vary in size from lymphocytes to the largest cells of the blood, which are slightly amoeboid and distinctly phagocytic, in fact, the chief phagocytes in malaria, sometimes containing a few pigment granules, sometimes many, these pigmented cells being almost as important in diagnosis as is the parasite itself.

They occur much of the time in the æstivo-autumnal form but only after the paroxysm in tertian and quartan. These phagocytic cells are said to rapidly become necrotic and to disappear from the circulation, which explains the diminution in the count at the end of an attack.

Stephens and Christophers cite, as illustrations of this relation of the leucocytes, the following cases: Bastianelli's fatal comatose case of æstivo-autumnal malaria, in which the small mononuclears were 19.1 per cent., the large mononuclears and transitionals, 41 per cent.; polymorphonuclear neutrophiles, 39 per cent., and eosinophile cells, 0.6 per cent. Panse's case, with a temperature at 37.2° C., and the small mononuclears, 18.1 per cent.; large mononuclears and transitionals, 26.4; polymorphonuclear neutrophiles, 55.3. In another case with temperature normal the small mononuclears were 14.8 per cent.; large mononuclears and transitionals, 46.7 per cent.; neutrophiles, 38.5 per cent.

In one of our tertian cases the total leucocytes were 16,500; large mononuclears, 38.3 per cent.; in a case of æstivo-autumnal, leucocytes, 6000, large mononuclears, 26 per cent.; in another, 4000 and 22 per cent.

A leucocytosis occurs rarely except in pernicious paroxysms. In one case in this clinic the count one hour before death was 50,000, of which the large mononuclears and transitionals were 18 per cent., the

polymorphonuclear neutrophiles, 58 per cent. It occurs also in malarial hæmoglobinæmia, in which case there is a marked increase in the polymorphonuclear neutrophiles, which begins with the attack and lasts for some time. A leucocytosis is seen also during the death agony, and is due to complications. There is a definite leucocytosis in the post-malarial anæmia, sometimes with increased eosinophiles and with myelocytes.

Bignami and Dionisi classify the anæmias of malaria as follows: Those which follow ordinary acute malarial fever, in which there is a secondary anæmia of a chlorotic type, without leucocytosis, with a few nucleated reds, leucocytes reduced, the large mononuclears relatively increased; (2) cases resembling primary pernicious anæmia, usually fatal, with extreme oligocythæmia, marked poikilocytosis, high color-index, nucleated reds if present for the most part megaloblasts, leucocytes diminished, and lymphocytes relatively increased; (3) rapidly fatal cases without any signs of regeneration which may have been in the first stages those of simple secondary anæmia. This anæmia is very similar to that which follows a severe hemorrhage.

(4) Chronic grave secondary anæmias of a chlorotic type and without nucleated reds; leucocytes much reduced. This is seen in a chronic malarial cachexia, and is due for the most part to degenerative changes in bone-marrow occurring after long infections, with the marrow sclerotic and pigmented (Thayer).

**Septicæmia.**—That septicæmia does not always cause a leucocytosis is seen in typhoid fever and acute miliary tuberculosis, and yet as a rule it does, but less constantly and markedly than do local pus accumulations. In the streptococcus and staphylococcus septicæmias, however, there is often a marked anæmia, with a greater and earlier fall than in any other acute disease. In one case of acute streptococcus septicæmia with hemorrhages (Grawitz) the cells fell to 300,000 in twenty-four hours. Septic fevers as a rule cause a loss of from 200,000 to 1,000,000 cells per week; that of puerperal infection has an especially bad effect upon the reds. The qualitative changes are marked,—degeneration, poikilocytosis, polychromatophilia, etc.; nucleated reds occur seldom in large numbers. The leucocytes vary as the patient's resistance, in some cases being very high, in other cases even subnormal.

We have had 26 cases of well-marked septicæmia. The final red counts in 15 fatal cases were from 1,000,000 to 2,000,000 in 2; 2,000,000 to 3,000,000 in 3; 3,000,000 to 4,000,000 in 4; above 4,000,000 in 6.

It is thus seen that the toxine does not kill through the anæmia (the drop in count after admission and before death was from 900,000 to 1,600,000), and that there are two groups of cases, those with high and those with low counts.

In 4 cases there was no leucocytosis at death. In 21 cases the leucocytes varied from 11,000 to 47,000. Some cases showed great variations in the white count. In one case with 8000 leucocytes 96.6 per cent. were polymorphonuclears; in another, of 10,400, 92.6 per cent. Gonorrhœal septicæmia caused a drop to 2,318,000, with 30 per cent. hæmoglobin, and at death leucocytes, 47,000.

**CHRONIC SEPTICÆMIA** it is important to diagnose, those cases of cryptic origin often passing unrecognized. In Ewing's case of em-

pyemia, with a duration of one year, the red blood-cells were 1,800,000, and the hæmoglobin 25 per cent. In other cases of pelvic abscess of even two years' duration the anæmia is slight.

We have had recently four cases of **Typhus Fever**:

CASE I.—Male, thirty-six years; red cells, 5,400,000; hæmoglobin, 72 per cent.; leucocytes on admission, 18,600; temperature, 103 to 104° F. On the eighth day after admission, 25,400; the temperature had begun to fall. Five days later, temperature normal; total count, 24,300; s. m., 3.2 per cent.; l. m. and tr., 6.6 per cent.; pmn. n., 90 per cent.; eosinoph., 0.2 per cent.

CASE II.—Male, nineteen years; red cells, 4,500,000; hæmoglobin, 70 per cent.; leucocytes on admission, 8600, and remained normal for four days; temperature, 102° to 105° F. On the fifth day, count 12,500; on the tenth day, with temperature normal, total, 10,000; s. m., 6 per cent.; l. m. and tr., 4.2 per cent.; pmn. n., 89.4 per cent.; eosinoph., 0.2 per cent.

CASE III.—Male, thirty years; red cells, 5,500,000; hæmoglobin, 85 per cent.; leucocytes on admission, 7000, and remained normal three days; temperature, 101° to 103° F. On the fifth day, 38,000; temperature, 98° F.; death (total count, 38,000; s. m., 5.8 per cent.; l. m. and tr., 1.2 per cent.; pmn. n., 93 per cent.).

CASE IV.—Male, twenty-two years; red cells, 5,400,000; hæmoglobin, 85 per cent.; leucocytes on admission, 9200, and normal three days; temperature, 102° to 104° F. On the tenth day they had risen to 11,600, but the temperature had already been normal three days. Leucocytes normal on the twelfth day (total, ninth day, 10,800; s. m., 15 per cent.; l. m. and tr., 11.5 per cent.; pmn. n., 72 per cent.; eosinophiles, 1.0; Matzellen, 0.8 per cent.).

From these four cases (daily counts were made in all) it is seen that the leucocytes are low on admission, even normal, then rise to a maximum, which occurs when the temperature has begun to fall or is already normal, then they fall to normal. (Compare with influenza and variola.)

This is different from the findings of Ewing and Thomas, who report absence of leucocytosis.

**Measles** and **German measles** have almost no influence on the red blood-cells, and cause no leucocytosis, or only a slight one. In the post-febrile stage the large mononuclears are increased.

Plantenga found in the thirteen cases of measles and the nine of Rötheln which he studied, a neutrophile hyperleucocytosis of even 20,000 during the prodromal stage, which rapidly gave place to a hypoleucocytosis during the eruptive stage, due to the disappearance of the neutrophile cells, and with sometimes a lymphocytosis and the disappearance of eosinophiles.

Renaud found in six cases that this preliminary leucocytosis reached its maxi-

mum about six days before the rash had appeared. This leucocytosis permits one to isolate a suspected case early.

Tileston could not confirm this leucocytosis during the prodromal stage, and thought all leucocytoses could be attributed to a complication.

We have very little material, but in nine recent cases in only one was the count above 8600 during the height of the fever (17,200).

**Scarlet fever** has very little effect upon the red blood-cells, but does cause a slight anæmia, the count averaging 4,500,000 (Reckzeh). The leucocytes are uniformly increased, an important point in diagnosis, rising during the incubation period, in some cases six days before the rash, and continuing elevated into convalescence and even until twelve days after the temperature has reached normal. This is an interesting exception to the rule of other diseases that the count runs roughly parallel to the temperature. They are generally normal on the fourteenth day (Reckzeh). The leucocytes vary from about 10,000 to 40,000; mild cases, 10,000 to 20,000; moderate, 20,000 to 30,000; severe, 30,000 to 40,000, according to the severity of the case and its duration. The neutrophile cells are relatively increased (to 85 to 98 per cent., especially in fatal cases); the eosinophile cells rapidly disappear and reappear with improvement; their failure to reappear is considered a bad sign. As a rule the eosinophiles reach their maximum two or three days after the rash appears, and are normal after the leucocytosis has disappeared. This early presence of eosinophilia is important in the diagnosis, excluding various septic conditions.

**Diphtheria.**—In this disease there is a moderate anæmia, a loss of about two million cells at the time of defervescence. During the height of the disease, however, there is often an increase in the count and specific gravity. (The injection of bacilli or their toxins into the circulation of animals causes a lymphagogue action which results in a hypercythæmia.) This hypercythæmia occurs most commonly in this of all the acute infections. In the case of Cutter the cells were from 7,200,000 to 7,800,000; in that of Morse from 5,000,000 to 5,500,000 during the first week, and 6,800,000 during the second. With the drop in the count the nucleated reds and the polychromatophilic cells appear. There is a slight leucocytosis—as a rule, 10,000 to 15,000, but in severe cases even 17,000, and with complications 30,000—which varies as the severity of the infection. The rise is in the polymorphonuclear cells. In some fatal cases there is leucopenia. The myelocytes are increased, especially in the fatal cases, to from 3 to 16 per cent. Morse says: “The examination of the blood in diphtheria is of no practical clinical importance in diagnosis, prognosis, or treatment.”

In ordinary follicular tonsillitis the counts are often as high as in diphtheria.

**Smallpox.**—“No other disease is so destructive to the red blood-cells” (Hayem). The anæmia is evident as the temperature falls, and



the question often arises whether it is truly due to a destruction of cells or merely to a dilution of the blood from the relaxed vasomotor tone. Yet during the pustular stage especially there may be a loss of 2,000,000 cells. Regeneration is slow, lasting about fourteen days. In the hemorrhagic form the anæmia is severe and varies with the amount of hemorrhage. Pick and Weil say that there is anæmia in the severe, none in the mild cases. Malassey says that the drop begins in the pustular stage, and that the rise begins with the desiccation or even during convalescence. The nucleated reds (normoblasts) are rare, except in the hemorrhagic form, in which they may be very numerous.

**LEUCOCYTES.**—From the onset with a normal count the blood formula is very characteristic of this disease. The polymorphonuclear neutrophiles are decreased, averaging about 40 per cent. or even 20 or 14 per cent.; the small mononuclears vary from 30 to 40 per cent.; the large mononuclears from 4 to 10 per cent.; myelocytes and irritation forms each 2 to 10 per cent.

The disease *per se* causes no leucocytosis, but during the pustular stage the leucocytosis is said to be the result of the infection by the skin cocci.

**Tuberculosis.**—Tuberculosis is a disease the virus of which can cause anæmia of the highest grade (*e.g.*, v. Limbeck's case of tuberculosis of the peritoneum and other abdominal organs, with a red blood-count of 730,000 and hæmoglobin 25 per cent.; such cases are so rare that this one is doubted by Cabot), but usually one of moderate grade, one often more apparent than real, and which may not exist at all. The degree of the anæmia is independent of the localization of the disease.

**IN TUBERCULOSIS IN GENERAL** a mild grade of chlorotic anæmia is the rule. This occurs in slight involvement of the apex ("anæmia of onset") without fever, in tuberculosis of bones and lymph-glands. This is the "pseudochlorosis tuberculosa." The count is almost normal, the leucocytes normal, and the hæmoglobin somewhat reduced. In other cases there is a lymphocytosis, absolute or relative; in some few cases a reduction of the count as well as of the hæmoglobin. Qualitatively some of the red blood-cells (not the majority, as in chlorosis) are rather pale and small; poikilocytes are usually few, may be numerous, but are rarer than in other cachexias of the same degree; nucleated reds are rare, even after a severe hemorrhage and when the anæmia is extreme, a point of importance in the differential diagnosis between this and carcinoma; Maragliano's endoglobular degeneration is seen in severe cases, especially in those of mixed infections.

Cabot considers that the tuberculous virus has itself but little effect on the blood, and that the above-mentioned changes are due to secondary infections, or to drains upon the proteid of the blood from diarrhoea, effusions, starvation, prolonged suppuration, etc.



In a pure case, with the exception perhaps of meningitis, the leucocytes are not affected. This is important in the diagnosis of peritonitis, bone troubles, and acute miliary tuberculosis. Qualitatively in some cases there is no change, and yet in others with a normal count is seen the increased percentage of the mononuclear cells common in all conditions with poor nutrition. If there is a leucocytosis, it is of the ordinary inflammatory type. The eosinophile cells are increased in some cases with cavity formations, and since this occurs also after the injection of tuberculin some think it is due to an auto-intoxication from the cavity. Myelocytes appear in advanced cases.

CHRONIC TUBERCULOSIS OF THE LUNG.—Grawitz has divided these cases into three groups: Group 1, with slight involvement of the apex, without fever, showing clear signs of anæmia, the pseudochlorosis tuberculosa, some with a normal count, others with a slight reduction, the leucocytes normal. Some early cases have almost normal blood. Whether there is a group in which the blood signs precede the physical signs may depend on the care with which the physical signs are sought. Group 2, cases of chronic phthisis with cavity formation but without other complications, the temperature slight; the blood picture is remarkably normal as regards count, hæmoglobin, specific gravity, dry constituents, yet there is with the cavity formation a general emaciation. Such a patient earlier had a distinct chlorosis; the leucocytes are normal or slightly increased, from 10,000 to 15,000 per cubic millimetre. Group 3, of cases with hectic fever, supposed by some to be due to a secondary infection but by others to pure infection with the tubercle bacillus. In these cases there is a true anæmia, a diminution in the count, sometimes rapid, of the hæmoglobin and dried substances, an anæmia which progresses often until death, with evidence of true blood destruction. The drop in the count may even be rapid.

In a recent case of pulmonary tuberculosis, two days before death the red cells were 1,473,000; hæmoglobin, 15 per cent.; leucocytes, 9000 (pmn. n., 88 per cent.; s. m. 5.9 per cent.; l. m. and tr., 4.7 per cent.; eos., 0.35 per cent.; normoblasts, 4, megaloblasts, 4 per 1000 leucocytes)

There is leucocytosis as a rule, especially if there is a secondary infection. In fact, v. Limbeck considers the presence of a leucocytosis sufficient guarantee of a secondary infection. Others disagree, for in the chronic septicæmic form there is a slight leucocytosis, and in caseous pneumonia as high a leucocytosis as in croupous pneumonia.

The normal count in the second stage has aroused considerable discussion. A concentration of blood by sweating, diarrhœa, and vomiting is not alone sufficient, although, if present, will help. Some consider the hypercythæmia a compensatory feature for the dyspnœa, since dyspnœa due to any cause produces a hypercythæmia;

others admit an anæmia which is covered by an oligæmia, and autopsies at this stage suggest a diminution of the total volume of blood. V. Limbeck considers the changed water metabolism the important point, the general drying of the tissues concentrating the blood; *i.e.*, an oligæmia vera. Grawitz considers the absorbed products of caseous nodules to have a lymphagogue effect, thus concentrating the blood.

After hæmoptysis the regeneration may be rapid (see page 579); after operation on a tuberculous focus if the hæmoglobin does not return rapidly the operation was probably incomplete; in some cases the anæmia accompanies the dissemination of the bacilli; in others the count rises and one can find no parallelism between the general condition of the patient and his blood; in fibroid phthisis, as a rule, there is no leucocytosis; in acute phthisis the anæmia is pronounced and progressive. In cases of cavity formation there is almost always a leucocytosis. In extensive tuberculous pneumonia some have little, others as high a leucocytosis as in croupous pneumonia. Acute miliary tuberculosis presents no change in the red blood-cells or hæmoglobin, and the leucocytes usually remain normal, but in a few cases are very low, even from 500 to 600 cells, over 90 per cent. of which are polymorphonuclear neutrophiles.

Tuberculosis of the serous membranes is accompanied by a mild secondary anæmia without leucocytosis unless the blood be concentrated by diarrhœa, except, perhaps, in some cases of meningitis, which is accompanied by a leucocytosis (Osler). In tuberculosis of the glands there is no leucocytosis until caseation begins. The injection of tuberculin into a tuberculous patient causes a leucocytosis with a rise of eosinophiles. In tuberculosis of the bones there is a marked absence of leucocytosis until a secondary infection sets in; a high count indicates abscess formation, but after the abscess has become chronic the count may remain normal until a secondary infection occurs. In these bone cases the reds are rarely diminished, but the hæmoglobin is low.

The very slight anæmia found in children is rather remarkable, since their blood is usually so susceptible. Brown,<sup>84</sup> in 73 cases, found the red blood-cells diminished only in the long-standing extensive cases in very young persons, but the hæmoglobin was diminished somewhat in all.

Of 17 cases of ACUTE MILIARY TUBERCULOSIS, in 5 cases the red cells stood between 3,600,000 and 4,000,000, and in 6 over 5,000,000. The color-index was quite low, in one-half the cases from 0.4 to 0.6. The leucocytes varied from 1000 to 9000, the majority (9) from 3000 to 6000.

One case had an interesting differential count (total, 3500; s. m., 6.5 per cent.; l. m. and tr., 10.8 per cent.; pmn. n., 81.9 per cent.; eosinophiles, 0.5 per cent.). Whartin's case with lower count, had 91.48 per cent. pmn. n.

Whartin reported a case with leucocytes often below 2000, and on one day (with a chill) 600, and Cabot a case with 550.

<sup>84</sup> Trans. Med. Soc. of the State of California, 1897, p. 168.

TUBERCULOUS MENINGITIS is an illustration of the general rule that the effect of an infection upon the leucocyte count depends partly on the location of the lesion, for tuberculosis of the meninges usually causes a leucocytosis.

Of 15 cases, in only 3 was the count below 10,000, and one of these was only a part of an acute general military infection. In the other two, one count each was made. The leucocyte curve is a very irregular one, 3 of our cases with high counts showing periods with low counts.

The highest count was 26,800.

In the series of 43 cases reported by Cabot there was leucocytosis in 32.

TUBERCULOUS PERITONITIS.—During the past four years we have had 22 cases. Of 19 cases in which the red cells were counted, 7 were between 3,000,000 and 4,000,000, and 6 between 4,000,000 and 5,000,000. The color-index varied from 0.5 to 1. Of the 22 cases there was a leucocytosis in 9 (highest count, 22,400).

Of Cabot's 60 cases there was a leucocytosis in 14.

TUBERCULOSIS OF BONES AND JOINTS.—These cases are reported chiefly from surgical clinics. There have recently been 15 cases in this clinic, with a leucocytosis in 6.

It is believed that during the active process of abscess formation there is a leucocytosis which in time will disappear, to reappear in greater degree if a secondary infection occurs; hence the high jump in the count which is a measure of the sepsis following operation, as a rule, this leucocytosis will soon subside, and so long as the abscess drains freely either not appear or the count will remain fairly low.

TUBERCULOSIS OF THE INTESTINE.—Of 5 cases there was a secondary anæmia in 2 (3,300,000 and 2,800,000 red cells). In one a leucocytosis of 14,000, which disappeared soon after admission.

Two cases of RENAL TUBERCULOSIS showed no leucocytosis.

One case of ADDISON'S DISEASE had red cells, 6,600,000; hæmoglobin, 92 per cent.; leucocytes, 9000. Sometimes there is marked anæmia in this disease.

**Typhoid Fever. RED BLOOD-CELLS.**—In the fresh blood smear may sometimes be found the very large phagocytic cells crowded with red blood-cells which Mallory has emphasized. Some find a slight rise of reds during the first week, then a slow fall to normal, and at the time of defervescence a true drop. Thayer<sup>85</sup> has reported the cases of this clinic. He found that from the end of the first week until defervescence there was a gradual reduction in the number of red cells, and that with defervescence regeneration began. In very long-continued cases the regeneration may begin slightly before the temperature is normal. The loss of reds averages 1,000,000 cells. The end of the third week is the average limit of the disease, at which time the average count of our cases was 4,555,814. The fall may be accentuated during the fourth week, and, indeed, the usual statement is that the anæmia begins at this time. During this period there are transitory variations in the count due to vomiting, sweating, diarrhœa, etc. Following a severe hemorrhage the anæmia is manifest, and regeneration begins at once.

Following some very severe cases is a post-typhoid anæmia, in one case with 1,426,000 red cells during the fourth week; in another

<sup>85</sup> Johns Hopkins Hosp. Rep., vol. viii.

case 1,300,000 during the third week (both Osler's cases), and one case of 804,000 (Henry). Usually there are no qualitative changes. After a hemorrhage are sometimes seen nucleated reds.

There is always a more marked reduction in the HÆMOGLOBIN than in the reds, the color-index, according to Thayer, varying from 0.7 to 0.8. In the above case with 1,300,000 cells the hæmoglobin was 20 per cent. The hæmoglobin runs parallel to the red blood-cells, but returns to normal more slowly.

The LEUCOCYTES, some think, are slightly increased at the very first, but apart from this they are subnormal during the whole course, and gradually diminish from the first (with 6400) to the fifth week, at which time the average of our cases was 5386. Some cases reach 2000, 1000 per cubic millimetre, or even lower. Thayer found no cases with an initial leucocytosis. The longer and more intense the infection the lower the leucocytes. In still other cases the count is above 10,000 throughout the whole course, cases without any complication. There may be temporary variations, the count rising to 10,000 cells after a cold bath, *e.g.*, yet with the differential count unchanged.

The *differential count* for the first five weeks shows a decrease in the percentage of polymorphonuclear neutrophils, usually to 60 per cent., and below 50 per cent. not rarely, and an increase of the mononuclears, especially the large, the transparent cells of Uskow, cells which are morphologically not lymphocytes, but which vary in size from these to the largest cells of the blood. They have relatively abundant protoplasm and faintly staining nuclei. These are especially numerous at the height of the fever. The eosinophile cells are reduced below 1 per cent., as a rule, until convalescence, when they increase to even above normal. They may, however, in long continued cases, increase with the increase in reds before the temperature is normal. During convalescence the count returns slowly to normal, but the blood retains its characteristic features for about three weeks after the temperature is normal.

The blood picture is modified by VARIOUS COMPLICATIONS. Hemorrhage causes an acute post-hemorrhagic anæmia with leucocytosis, the lowest count of our series being 1,992,000 cells; regeneration begins at once, and the cells are usually restored in a little over one week.

The inflammatory complications are accompanied by a rise, or even a true leucocytosis. This is true of furunculosis, phlebitis, thrombosis, bronchitis, periostitis, pleurisy, pneumonia, etc. A definite rise in the count, already very much reduced, is for that person often a true leucocytosis; for instance, in one case in our wards the leucocyte count accompanying parotitis rose from 1600 to 3200 cells, comparable to a rise in a normal person to about 15,000 cells.

In one case of empyema the count was 44,500; in a second case, due

to the typhoid bacillus, 23,000 cells, of which 68.5 per cent. were polymorphonuclear neutrophiles; small mononuclears, 12.7 per cent., and large mononuclears, 17 per cent.

Of the 5 cases of pneumonia of our series, 3 cases, of whom 2 died, had counts above 10,000 cells; 2 cases, both of whom died, below 10,000 cells. In all of these cases the differential count showed a smaller percentage of the polymorphonuclear neutrophiles than one would expect. In periostitis due to the typhoid bacillus the leucocytes in one case were 18,000, of which 72.5 per cent. were polymorphonuclear neutrophiles. Thayer cites many similar illustrations showing that in an inflammation due to the typhoid bacillus the reaction of the blood depends more upon the situation of the infection than upon the organism, and the tendency, as illustrated by the above cases of empyema and periostitis, is for the formula of typhoid fever to persist.

**PERFORATION.**—In our cases of suspected intestinal perforation the leucocytes are followed with the greatest care, and interpreted as in appendicitis. From the onset of the first symptom, whether it be abdominal pain, hiccough, or any other feature which points to the abdomen, the leucocytes are counted each hour. If the abdominal features are suggestive of perforation, the operation is performed, whatever the leucocytes may show. If, however, there is a rising count, an operation is performed although the local signs may seem insufficient. It is granted that a rising leucocytosis may mean something else than perforation. In one of our cases it was appendicitis. In this clinic practically every case in which either of these features is present is operated upon, under the belief that it is much safer to operate too soon than too late, and that an unnecessary operation affects very slightly the course of the typhoid fever. We have succeeded in this way in saving about 30 per cent. of our cases of perforation. In most cases there is a slight rise of the leucocytes, either an absolute leucocytosis of 10,000 or over, or one relative to the previous counts. Following this rise is sometimes a drop which some suppose is coincident with the spread of the peritonitis. In some malignant cases occurs a fall without any preliminary rise. In those so-called pre-perforative cases there is a slight leucocytosis due to the local peritonitis. While a great deal of weight is placed upon the leucocyte count no absolute value is allowed it, and it is always interpreted in the light of the physical examination.

**Pneumonia.**—The coagulation is rapid, as a rule. The count of the *red blood-cells* is normal during the fever, or there is a rise at first, as in Sadtler's case to 7,000,000. After the crisis there is always a drop of about 500,000 cells, sometimes a slight post-febrile anæmia. The hypercythæmia is probably due to the concentration of the blood. This often covers a real anæmia caused by the loss of blood to the exudate

and by the destruction of the blood-cells, as shown by the jaundice and the urobilinuria. Cases with these two features show a greater anæmia than others, with a loss of about 2,000,000 cells. The drop which occurs on the day of crisis is partly the disappearance of the hypercythæmia and a drop below normal due to the general peripheral relaxation (Grawitz). In addition to this is a certain grade of true anæmia which sometimes is severe.

Nucleated reds are more common in pneumonia than in other acute fevers. Both normoblasts and megaloblasts occur, which latter have a bad prognostic import only when present in considerable number. At the time of the crisis it is thought the cells crenate more easily than normal, evidence of some chemical irritant in the blood.

In 34 cases in which special attention was paid to the red blood-cells there occurred a drop during the lysis or just after the crisis, generally of about 1,000,000, but in some cases of 2,000,000 cells, which drop usually only restored the count to that level which obtained before the hypercythæmia. The later counts showed small gains and losses in an even number of cases and of about the same degree, but in 9 cases there was a permanent loss of from 900,000 to 1,500,000, and in 4 cases a gain of from 700,000 to 1,900,000.

Pneumonia is the disease in which the *inflammatory leucocytosis* has been best studied. None of our cases showed evidence of an initial hypoleucocytosis, as claimed by Pick. From the first, six to eight hours after the chill, the leucocytes are found increased, and they drop at about the time of the crisis. This leucocytosis is an expression of the resistance of the organism to the infection, and depends but little on the fever and the extent of consolidation. Cabot has divided the cases into three groups: (1) Those with good resistance and a mild infection, in which there may be no leucocytosis; these cases all recover. (2) Those with a severe infection and a good resistance, in which the leucocytosis is high, between 20,000 and 30,000, but in some cases over 100,000, even 115,000 (Löhr). This group includes about 90 per cent. of all cases. (3) Those cases with a poor resistance but a severe infection, in which there is no leucocytosis or even a fall, and which cases are almost always fatal. The best illustrations of this last group are the terminal pneumonias of chronic diseases, pneumonia in the aged and in alcoholics. In fatal cases in which the count does not rise the percentage of polymorphonuclears may rise considerably. In case of a pseudo-crisis the statement is made that the leucocytes do not drop, and even rise. The fall in the leucocytes begins just before, just after, or with that of the temperature, and may be preceded by the maximum count. Löper claimed two maxima, the one at onset, the other just before the fall. They fall by lysis rather than by crisis, reaching normal on about the second day. If the temperature falls by lysis, the leucocytes fall as a rule more slowly. In cases in which a slight temperature continues

after the crisis the leucocytes remain elevated until this returns to normal. In fatal cases there is often an ante-mortem rise. In delayed resolution the leucocytes may stay elevated even for weeks and then slowly drop, but in these cases the temperature and leucocytes usually become normal at the same time. For the count to remain elevated suggests delayed resolution, empyemia, or gangrene.

A high count gives no idea of prognosis; it means that the patient is making a vigorous fight, but gives no hint as to which will win, he or the infection.

In our pneumonia cases the leucocytes are counted twice daily. We have compiled the records of some of these cases (158 uncomplicated cases with recovery, 56 uncomplicated cases with death, and 80 cases with various complications), studying them as regards age and termination. In our uncomplicated cases with recovery sex, and the extent of the consolidation have no relation to the degree of the leucocytosis. Age has remarkably little influence; exactly the same percentage of cases below forty years had a leucocyte count below 20,000, as of those older. In all the uncomplicated cases with recovery, 38 per cent. were below 20,000; 7 per cent. above 40,000.

Seventy-seven of these cases terminated by crisis. Of these the cases above 40 years of age had an average leucocytosis somewhat higher than those younger, probably since there were fewer low counts. With 10,000, were no cases; from 10,000 to 15,000, 18 per cent.; these were clinically very mild cases; from 15,000 to 20,000, 25 per cent.; above 40,000, 8 per cent. There was a sharp rise at crisis in 42 per cent. of the cases.

Of 81 cases with lysis the count during the course was below 10,000 in 2 per cent.; from 10,000 to 15,000 in 20 per cent.; from 15,000 to 20,000 in 14 per cent.; above 40,000 in 10 per cent. There was a sharp rise just at lysis in 34 per cent. These rises, which occurred just before the lysis or crisis, were of from 5000 to 10,000 cells, as a rule, but in a few over 20,000, and in one case 30,000. The highest count during the course was 105,500—a young man 25 years old who recovered.

Of the cases with crisis the leucocytes began to drop before the temperature in 15 per cent.; with the temperature in 41 per cent., and after it in 44 per cent. In the lysis cases the drop began before the temperature in 18 per cent.; with, in 43 per cent., and after, in 39 per cent., which are practically the same figures as for those cases with crisis. To reach normal required in the crisis cases from one to twenty days, but the mean time was three days. A well-marked pseudocrisis occurred in 9 cases; of these, 2 were accompanied by a rise of leucocytes, 4 by a fall, and 3 by no change. In cases with a slight fever for some days after the drop the leucocytes remained from 12,000 to 15,000 until the temperature reached normal.

There were 56 fatal cases. The leucocyte counts in these were almost the same for the various decades as in those with recovery. During the course they remained below 10,000 cells in 23 of the cases (in one case they reached even 1700); from 10,000 to 15,000, 23 per cent.; from 15,000 to 20,000, 15 per cent.; and over 40,000 in one case. At the time of death the count was below 10,000 in 17 per cent.; from 10,000 to 15,000 in 25 per cent.; 15,000 to 20,000 in 10 per cent., and above 40,000 in 8 per cent.; all the last cases were under thirty years of age. Toward death in 70 per cent. there was a progressive rise, in 30 per cent. a fall. The absence of leucocytosis is not necessarily fatal. In one case with extreme toxæmia and a count of 8000 the leucocytes slowly rose to 14,000 as the patient recovered.

**DAILY VARIATIONS IN THE COUNT.**—The two counts were made in the forenoon and afternoon, and separated by an interval of about nine hours. These counts differed by from 1000 to 26,000 cells, as a rule from 4000 to 6000, with a mean of 4000. There was no difference in these variations before and after the crisis. When the temperature was very constant the variation was less marked, the



greatest variation occurring in cases with an irregular temperature, but even then there is no parallelism between fluctuations of temperature and leucocytes, in some cases even a reverse relation.

In cases of delayed resolution, in some the leucocytes reached normal before the temperature; in others both temperature and leucocytes were normal before resolution was complete; again, in others the temperature was normal before the leucocytes.

The cases of terminal pneumonia vary much, our series showing two with counts above 50,000 and two below 3500. Alcoholics had almost no leucocytosis, and yet some recovered. In all cases followed by empyema the leucocytes for the most part showed no change which would indicate when the resolution or the empyema began. In one case throughout the whole disease and to the time of the operation there was no leucocytosis, and in another case the leucocytes did not rise at all until the empyema began. In 2 cases followed by pleurisy with effusion the leucocytes were normal after the crisis (6000 and 8000). In 3 fatal cases ending in abscess of the lung the leucocytes were respectively 46,000, 30,000, and 8500. In 35 cases with various pus infections, endocarditis, pericarditis, meningitis, parotitis, otitis media, phlebitis, thrombosis, tonsillitis, etc., very little could be learned from the leucocytes; that is, there was no notable rise, although the fall may have been delayed. If already low, they did not rise.

*Qualitative Changes.*—The leucocytosis is of the polymorphonuclear variety, and yet the percentage of the polymorphonuclear neutrophile cells is seldom 90 per cent., often not over 80 per cent. After the crisis they drop to 60 per cent. and even below. The small mononuclears show an absolute increase and a relative decrease. The eosinophile cells may actually disappear during the height of the attack. They reappear at the crisis, but not in large numbers. At the crisis many myelocytes may appear, even 12 per cent. of the total number of white cells, and the large basophile mononuclears may be much increased. For the polymorphonuclear neutrophiles to be above 90 or below 50 per cent. is considered to indicate a bad prognosis.

*Glycogen* can nearly always be demonstrated in the leucocytes, in amount varying with the temperature and the extent of consolidation.

The *platelets* may even disappear during the continued fever, but after the crisis increase to above normal.

The *fibrin net-work* is increased more than in any other disease. Coagulation is rapid. *Specific gravity* varies as the count and is high. The toxicity of the blood is even double.<sup>86</sup>

In a doubtful case the presence of a leucocytosis excludes malaria and typhoid fever and suggests a central pneumonia. This is especially important in the very old and in the very young patients, and in cases without localizing symptoms.

In mild cases of the BRONCHO PNEUMONIA OF INFLUENZA there is a polymorphonuclear neutrophile leucocytosis of from 10,000 to 15,000 and in severe cases of from 20,000 to 25,000 cells per cm.\*

In *Intestinal Parasites* a slight leucocytosis is the rule. In 12 of

<sup>86</sup> Albu, Virchow's Arch., vol. cxlix.

\* Davis, Arch. Int. Med., 1908, vol. ii, p. 124.



our 18 recent cases these cells varied from 11,200 to 34,000. In 4 of the cases with normal counts there was some fever.

**Bronchial Asthma.**—In bronchial asthma the most interesting find is an eosinophilia of even 53.6 per cent., which is important in diagnosis, and by means of which the oncoming paroxysms may in some cases be predicted.

In 17 cases during the past four years the red count was high, over 5,500,000 in 7 cases; the lowest, 4,900,000. There was a leucocytosis of 10,000 to 15,700 in 6 cases. In but 8 cases were differential counts made, but of these, 6 had above 5 per cent. eosinophiles; maximum, 20 per cent. in a total count of 8600. (Of these six, the absolute numbers of eosinophiles were 728, 712, 535, 856, 702, and 1720.)

**Acute Articular Rheumatism.**—"The blood is the best index of the severity of this disease" (Osler). Its virus is a rapid and powerful destroyer of the red cells, causing often, but not always, a reduction of from 1,000,000 to 2,000,000 cells. Ewing considers that the anæmia has been exaggerated. The high count which is sometimes seen during the attack may be due to the sweats. This concentration of the blood may cover the anæmia, which often is most evident at the time of convalescence. Hayem, Türk, and others say that the count is lowest at the height of the fever, and that regeneration begins at once with defervescence. It is rare to find nucleated reds. The hæmoglobin suffers worse than the red blood-cells. In no other disease is the fibrin net-work so thick.

Leucocytes are increased, as a rule their count running parallel to the severity and acuteness of the disease. Cabot's average was 16,000. Changes in the differential count are those of other acute diseases. In subacute or chronic rheumatism there is no leucocytosis.

Of 77 cases of this disease, the red counts were, 2,000,000 to 3,000,000, 3; 3,000,000 to 4,000,000, 15; 4,000,000 to 5,000,000, 45; 5,000,000 and over, 14; mean count, 4,500,000; hence very little anæmia. Of 81 cases, the leucocytes were below 5000 in 1 case, from 5000 to 10,000 in 23, 10,000 to 15,000 in 36, 15,000 to 20,000 in 15, above 20,000 in 6.

Another case, a man fifty-six years of age, was admitted with red cells, 1,720,000; hæmoglobin, 27 per cent.; leucocytes, 12,400. He gave the history of painful, swollen, red joints four weeks before. He recovered rapidly.

**Arthritis Deformans.**—McCrae, in 33 cases, found the average of hæmoglobin 70.6 per cent.; of reds (29 cases), 4,468,000; the leucocytes, 7600; the differential count normal.

**Appendicitis.**—In acute appendicitis the rule which our surgeons follow is the same as for all acute abdominal cases. After the first suggestive symptom, or on admission, the leucocytes are counted each hour. With a rising leucocytosis an operation is performed without delay, even though the abdominal signs are very slight; on the other hand with marked abdominal signs the operation is performed, what-

ever the leucocytes may be. If the leucocytes are high but stationary when the patient is first seen, one can wait; but if rising, even slightly, there should be no delay. A normal count means nothing; the case may be mild, very severe, or a well-walled abscess. A high leucocytosis, 20,000 or above, indicates acute appendicitis, probably an appendix full of pus and quite tense. The leucocytes probably fall after it ruptures, at least those cases which have recently ruptured are admitted with low counts or even a subnormal count, even while the process is spreading. In appendicitis 20,000 is a high count, and means pus, gangrene, or peritonitis; above 15,000 means an active process. In fulminating cases there may be death without any reaction on the part of these cells.

In chronic appendicitis with abscess a stationary leucocytosis means a well-walled abscess. In those cases with old abscess the count is seldom above 12,000, and often from 6000 to 7000, but after the operation on such a case the leucocytes will rise at once to about 20,000 or over and then gradually drop. This is perhaps due to exposure of a new area to the infection. If the count remains high it means a pocket is still unopened. While many of our cases with well-walled abscess show a normal leucocyte count, yet these cases also show marked fluctuations for which no explanation is offered. In those cases in which the physical features indicate a rupture of the abscess and a spreading peritonitis, the count may rise or drop, or may fall even to subnormal, or, after a fall, may then again rise, the falling leucocytosis being a worse sign than a high stationary.<sup>87</sup>

In catarrhal appendicitis, and chronic appendicitis without any exudate, there is no leucocytosis.

The red cells show no change except in cases with long standing abscess, in which there may be a secondary anæmia. Da Costa mentions an early slight anæmia in most cases, in some a severe anæmia.

**Anæmias of Children.**—The study of this subject is of especial importance, since the blood picture is often so different from that in the adult. We have even known a diagnosis of lymphatic leukæmia suggested when the blood was normal.

Children are much more susceptible to all agencies causing anæmia than are adults; the anæmia is more rapid in development, more severe, less easily recovered from. The narrow changes are much more strikingly mirrored in the blood, and when regeneration does begin the picture is quite spectacular, normoblasts, megaloblasts, and myelocytes appearing in such good numbers.

The "*anæmia of growth*," it is claimed, is explained by the inability of the blood-building organs to keep pace with the growth of the body. At this time wasting diseases, infections, or any agency del-

<sup>87</sup> See Bloodgood, *Prog. Med.*, December, 1901.

terious to the blood, vascular system, or the hæmatopoietic organs, as poor food, bad hygienic surroundings, influence the child much more than the adult. The development of the heart and of the whole vascular system is also in the closest relation with the blood condition, hence chlorosis and other severe anæmias of youth occur in children with hypoplasia of the cardiovascular system, and this is attributed to a congenital defect. The anæmia of school-children is due to mental strain, lack of exercise, poor appetite, constipation, etc.

A long-standing anæmia in a child is perhaps never perfectly recovered from. Objective evidence of it may disappear and the child seem well, but relatively insignificant agencies will cause it to reappear.

In all such anæmias of the very young a lymphocytosis is usually present and unripe elements appear—normoblasts, myelocytes, and large basophilic non-granular leucocytes—which do not occur in the normal blood. But these qualitative changes in the blood picture have less significance than in the adult, and show more an instability of the blood-regulating mechanism.

The ANÆMIA PSEUDOLEUKÆMICA INFANTUM of v. Jaksch was described as a condition in young children of severe anæmia (even 820,000; most were 1,500,000 to 3,500,000), with low color-index (0.50), a leucocytosis of even 54,660 (in one case, 114,000), with a few myelocytes.

Among the red cells are sometimes many deformed and degenerated ones, and many nucleated reds. The leucocytes are characterized by their great variations in form, size, and staining qualities. The platelets are increased.

The best recent discussion of this condition is that of Cabot,<sup>88</sup> who thinks that the many very different cases thus diagnosed cannot be grouped together. They might be cases of pernicious anæmia, secondary anæmia with leucocytosis (due to lues, rickets, etc.), or Hodgkin's disease, or either of the leukæmias, all of which diseases are apt to be atypical in children.

Bezançon and Labbé emphasize inherited lues as the cause.

Reckzeh, from analogy from experiments on adult and young dogs, considers this form in children a simple anæmia,—that the special features differing from that of an adult are due to the reaction of the young.

*Malaria* in children causes an especially severe anæmia, with normoblasts and perhaps always megaloblasts, but no marked leucocytosis except perhaps a lymphocytosis.

*Congenital lues* causes the severest anæmia, with many nucleated

<sup>88</sup> Fifth edition, "Clinical Examination of the Blood," p. 519.

reds and the lymphocytes much increased, the total count being even from 50,000 to 100,000.

*Rickets* causes a simple chlorotic anæmia, with the leucocytes even 30,000, most of them small mononuclears.

A recent case of anæmia in a fourteen-months-old child is entered on our records simply as "Anæmia with enlarged liver and spleen" (Osler). The red cells on admission were 1,252,000 per cubic millimetre; hæmoglobin, 20 per cent.; leucocytes, 14,700. The child was in the ward one month, and did not improve at all. The leucocytes varied from 13,000 to 26,500, always with the same formula (s. m., 40 to 52 per cent.; l. m. and tr., 5 to 18 per cent.; pmn. n., 38 to 62 per cent.; eos., 0 to 0.9 per cent.; neutroph. myeloc., 0.9 to 3 per cent.; Mastzellen, 0 to 0.2 per cent.; nucleated reds, 24 to 250 per 1000 leucocytes, chiefly normoblasts, some intermediates, and megaloblasts and microblasts).

Such a case resembles the French "splénomégalie chronique avec anémie et myélemie."

**SUMMER DIARRHŒAS OF CHILDREN.**<sup>89</sup>—The ordinary summer diarrhœas are usually accompanied by a leucocytosis, but of so wide a range that it has no diagnostic value. In the simple dyspepsias the differential count of leucocytes is normal (total, 13,500 to 36,000; s. m., 39; l. m. and tr., 21.2; pmn. n., 37.8; eos., 2), but in the more severe cases there is an increase in the polymorphonuclear neutrophiles (56 to 63 per cent.), a decrease of the mononuclear cells (33 to 7 per cent.), the blood thus presenting the picture of an adult blood. In an acute intestinal toxæmia and the severe forms of enterocolitis occurs a true leucocytosis.

A leucocytosis with a wasting disease in a child usually indicates an inflammatory intestinal complication.

Myelocytes are few, eosinophiles sometimes disappear. The leucocytosis is no indication of the severity of the disease, but the percentage of the various cells is.

In some cases of severe diarrhœa the red count may rise even to 10,000,000 cells.

#### CHRONIC DISEASES

**Chronic Nervous Diseases.**—These present nothing at all characteristic in the blood; in these diseases its condition depends more on the general nutritional condition of the patient.

Some cases of CHOREA are very anæmic, but the chorea is probably not the primary condition. In 23 cases of this clinic there were but three with a mild secondary anæmia, the lowest 3,400,000 reds, and these were also heart cases.

There is sometimes an eosinophilia of from 7 to 10 per cent. (Thélème).

In GENERAL PARESIS Capps and Jenks<sup>90</sup> found in some cases just

<sup>89</sup> Knox and Warfield, Johns Hopkins Hospital Bull., July, 1902.

<sup>90</sup> Am. Jour. of Insanity, January, 1900; Diefendorf, loc. cit., 1903, vol. cxxvi.

before a paretic seizure an absolute leucocytosis, with an increase especially of the large mononuclears. In this condition is also some anæmia which progresses with the disease, except during the seizure, when is seen a temporary rise of reds.

In MANIACAL DEPRESSIVE INSANITY Fisher<sup>91</sup> found often an anæmia, almost always a leucocytosis during the periods of excitement, but no pathognomonic blood-changes.

In ACROMEGALY there is an increase in reds with eosinophilia and lymphocytosis (Ducati).

**Diabetes Mellitus.**—The symptoms of this disease are essentially those on the part of the blood, and the urinary ones are only secondary, the glycosuria being the result of the hyperglycæmia, the sugar in the blood reaching even 0.57 per cent. instead of, as normally, 0.1 to 0.2 per cent.

Concerning the red blood-cells the reports are various. The hyperglycæmia causes an hydræmia, and hence dilution of the blood; diuresis, on the other hand, concentrates the blood, hence the actual count found varies considerably. Later in the disease, however, as the cachexia develops there is an anæmia, and yet this anæmia may be well masked in a concentrated blood. The leucocytes are normal; the patients show a remarkable digestive leucocytosis.

In 45 cases, the red cells were below 4,000,000 in 3 cases; the lowest was 2,000,000; from 4,000,000 to 5,000,000, 13 cases; 5,000,000 to 6,000,000, 19 cases; over 6,000,000, 4 cases. Three other cases were at times over 6,000,000.

Of 40 cases, the leucocytes in 25 varied from 5000 to 10,000; from 10,000 to 20,000 in 7 cases; over 20,000 in 7; the highest, 44,000. The explanation of the leucocytosis was pneumonia, septicæmia, furunculosis, gangrene; and in a case of coma they varied from 30,000 to 41,000.

LIPÆMIA is common especially in severe cases. In this condition the serum is milky from the increased fat in the blood, this being present in dust-like particles, which are sometimes coarse; the fat is increased greatly above the normal amount. Bönninger found 0.75 to 0.85 per cent.; normal, 1 to 3.25 p.m.; others in diabetes from 30 to 117 p. mille; yet lipæmia may exist with but 5.5 p.m., and we cannot claim an exact relation between the visible and total fat. Lipæmia is physiological in sucklings, in very obese persons, in some pregnant women, and in adults after a heavy fat meal; but the best examples are severe cases of diabetes mellitus with considerable sugar excretion, even after a fast of twenty-four hours. It may exist for several weeks before death.

The cause is perhaps disturbed oxidation of the fat present, probably the fat of the diet, while others claim the sugar is directly transformed to fat. Lipæmia occurs also in cases of chronic alcoholism,

<sup>91</sup> Am. Jour. Insan., April, 1903.

pneumonia, in progressive tuberculosis, in fracture of long bones, in contusions of subcutaneous fat, and, it is said, in some cases of liver and kidney disease, malaria, cholera, and phosphorus and carbon monoxide poisoning. Fitcher<sup>92</sup> has contributed a recent article on lipæmia, and Fraser<sup>93</sup> has reported an interesting case with 16.44 per cent. of fat in the blood and 18.94 per cent. in the pleural exudate. The record case is Fischer's, with 18.129 per cent. in the blood.

BREMER'S BLOOD-TEST has proved valuable in certain cases. A thick smear of blood is made on a slide, and a similar one for a control of normal blood. These are subjected to exactly the same treatment. They are first heated to 135° C., then allowed to cool slowly, and are stained with 1 per cent. Congo red, aqueous solution, for two minutes. Relative to the normal blood the diabetic blood will take a yellow rather than a red tint. This test is positive also when the urine is sugar-free, and is said to be given even before sugar has appeared. Schneider found it present in two normal men who were great meat eaters, and ascribes it to the reaction of the blood. Strauss confirms this opinion, finding it best in cases of acidosis. It is claimed to be sometimes present in cases of leukæmia, Hodgkin's disease, and Graves's disease, and yet, granting this, it is of importance in diagnosis.

In this clinic a man was admitted during coma; no urine could be obtained by catheterization; the diagnosis of diabetic coma was made from this test alone, and confirmed later by autopsy.

WILLIAMSON'S TEST.—Twenty cmm. of blood in a test-tube are mixed with 1 cc. of aqueous methylene blue (1 to 6000); 40 cmm. of 60 per cent. KOH and 40 cmm. of water are added. This mixture is allowed to stand for three or four minutes in boiling water. If the blood be diabetic it takes a yellow color.

QUANTITATIVE DETERMINATION OF FAT IN THE BLOOD.—The method chosen by Bönninger, in Salkowski's laboratory,<sup>94</sup> is as follows: From 5 to 30 gm. of blood are mixed with 10 to 20 volumes of 96 per cent. alcohol, the precipitate ground fine, then allowed to stand one or two days. It is then filtered, the precipitate again extracted several times with alcohol in the same way, then with 5 to 10 volumes of ether twice, digesting one day each time. All these extracts are then combined, evaporated, repeatedly taken up in absolute alcohol, and this evaporated, then filtered, dried, and weighed. Extracting twice with alcohol alone will give 96 per cent. of the total fat.

**Malignant Disease.**—Malignant diseases are the most important anæmia-producing diseases. The immediate cause of the anæmia may be the frequent hemorrhages, the mechanical effects of the cancer, and this is well seen in gastro-intestinal cases, and, most important, the cancer toxine. That the toxine is the most important factor is shown by the extreme anæmia caused by a small latent nodule which can cause no local symptoms. The result of the growth is an anæmia

<sup>92</sup> Jour. Am. Med. Assoc., October 21, 1899.

<sup>93</sup> Scot. Med. and Surg. Jour., 1903, p. 200.

<sup>94</sup> Zeits. f. klin. Med., 1901, vol. xlii.

parallel to the progressive cachexia. And yet it is remarkable how long the blood will present an almost normal picture before the slight anæmia begins, and then how rapidly the anæmia and cachexia will develop. Grawitz claims that the cancer produces a plasmotropic poison; that is, one which may affect the blood, or the blood plus the body tissues, or the tissues without the blood, producing in some cases merely degenerations of the red blood-cells; in other cases an anæmia parallel to the cachexia; in still other cases a marasmus of the highest grade yet with the blood only slightly affected.

Cancers vary much. Some, for instance those of the skin or lip, cause no anæmia, while a fulminating cancer, as of the stomach, "may give a perfect picture of primary pernicious anæmia, or, indeed, of leukæmia." In general it is stated that the more malignant the tumor the greater the blood changes, and the more extensive the cancer, that is, the more its metastases, the greater its influence upon the blood. But this is not entirely true: our cases with rapidly developing metastases, with large nodules, are those with a slight chlorotic anæmia; those which simulate pernicious anæmia are more often cases with few if any objective signs of cancer, and at autopsy one finds an insignificant looking little nodule.

The common picture is the so-called "pseudo-chlorosis carcinomatosa;" others show the picture of the severest pernicious anæmia, the cases being thus diagnosed, and the diagnosis corrected at the autopsy by a small cancer nodule, often of the stomach, which before that was unsuspected. In still other cases no anæmia results. Cabot says in one-half the cases there is a chlorotic anæmia; in about one-fourth, none; while one-fourth show a reduction in both count and hæmoglobin.

The severest anæmia occurs in those cases with frequent hemorrhage, as, for instance, of the stomach and of the uterus. Anæmia from cancer of the digestive tract is sometimes great because of the resulting malnutrition. v. Limbeck says that it is common, and perhaps the rule, to find even in advanced cases blood which is qualitatively normal; that in cases with dessicated tissues there may be even a rise in the count; and in advanced cachexia cases without hemorrhage there is seldom much diminution in the red count.

The anæmia when it does occur is of the secondary type as a rule, and severer than that due to any other chronic disease. The chief changes are at first in the size, shape, weight, and degeneration of the red blood-cells; later, as the cachexia develops, the anæmia is often as low as 2,500,000 cells, or even as low as in pernicious anæmia, 1,000,000 cells. An exception to this is in cancer of the œsophagus, where, indeed, the blood may be concentrated.

There is a constant and often an early reduction in the hæmoglobin. Its percentage may be normal but then the count will be found above normal; the average in long-standing cases is about 68.5 per cent., in worse cases 57.5 per cent. It is, therefore, rarely as low as in chlorosis. This low hæmoglobin is an important diagnostic point between malignant and non-malignant tumors. Cabot's average was 58 per cent., with an index of 0.65. The hæmoglobin is lower in cases of visceral than peripheral cancer, and the index is low even in severe cases; yet Bezançon and Labbé mention two cases in which the cells were increased in size and with an index over 1. After operation regeneration begins at least one week later than would be expected, and it is said never quite to regain the percentage it was before operation, even though the patient gains in weight (Bierfreund).

There is always diminution in the average size of a few or most of the cells. The giant cells of pernicious anæmia are rarely seen here except late, while microcytes are common (Grawitz). The basophile granulation is very common. The deformities in size and shape and all degenerations may be absent or they may be even more marked than in tuberculosis, in which case they are of diagnostic value; they are a less prominent feature, however, than of pernicious anæmia. Nucleated reds are present as a rule and always in the severe cases. They are more numerous than in secondary anæmias due to any other cause, and are found even when the anæmia is slight. Their presence has some diagnostic value in a differential diagnosis between cancer and ulcer of the stomach. They are normoblasts, as a rule, although in those cases which simulate pernicious anæmia a few megakaryoblasts may be present. In cases involving bone-marrow their number may be enormous, even 90,000 per cubic centimetre (Türk).

The blood is hydræmic, with reduced albumin. Metabolism experiments indicate an abnormal destruction of tissue proteid, the evidence of a circulating toxine. Grawitz suspects that in some cases the anæmia is only apparent, since the injection of carcinoma extract shows that the tissue lymph will dilute the blood. But this others doubt. Much of the anæmia is due to repeated hemorrhages, which are common enough, yet there is all the evidence necessary of a hæmolytic toxine.

**LEUCOCYTES.**—In general, in about 60 per cent. of all cases, there is a moderate leucocytosis, an important point in the differentiation of benign and malignant tumors, and this may be the first sign of cachexia. Of some the count is normal; some present the appearance of leukæmia. This leucocytosis depends upon the hemorrhages and the position of the cancer. In œsophageal stricture the starvation will sometimes cause a leucopenia, and the cells present will be chiefly lymphocytes; in cancer of the uterus and stomach so commonly accom-



panied by hemorrhage, a post-hemorrhagic leucocytosis is common; in those of the thyroid, pancreas, and kidney the count is especially high. It also depends upon the size of the cancer, including of course the metastases; the larger and faster the tumor grows the more the leucocytosis; but there are great variations. Grawitz, from his injection experiments with cancer extract, considers that the increase is due to the entrance of the tissue lymph into the blood-stream, carrying with it a great many leucocytes, the same which occurs in a post-hemorrhagic leucocytosis, and hence considers that the leucocytosis is coincident with the softening of a tumor mass. After operation the leucocytes drop, and their subsequent rise may indicate a recurrence even before it can be found physically.

**DIFFERENTIAL COUNT.**—As a rule, a leucocytosis means an increase of the polymorphonuclear neutrophiles, but in some cases these are low, even 43.7 per cent., and the lymphocytes are increased. In other cases there is a leucopenia of even 3000, but with the polymorphonuclear neutrophiles even 88.7 per cent. The eosinophiles are not usually as much diminished as in other leucocytoses, but on the other hand, they may not be increased even when bone metastases are present. Myelocytes are more commonly present in cancer than in any other condition excepting leukæmia and pernicious anæmia.

The specific gravity of the blood is low. The plasma is rich in sugar, even as rich as in diabetes. Its alkalinity is decreased even to one-third. The coagulation is normal or retarded unless sloughing or inflammation be present, in which case it may be rapid. The fibrin net-work is usually normal.

It is said that the effect of cancer is seen in degenerative changes of the leucocytes before the quantitative changes begin.

In **CANCER OF THE BREAST** a slight leucocytosis (11,000) is common. In **CANCER OF BONE** are found very many nucleated reds, normoblasts and megaloblasts, and leucocytosis with a high percentage of mononuclears, and some myelocytes.

**CANCER OF THE STOMACH.**—A cancer of the stomach may cause a blood condition which resembles closely primary pernicious anæmia. In these cases the red cell count may be very low, even 500,000 per cubic millimetre (Grawitz). But such cases are rare. Cabot found that of 129 cases, in 27 the count was above 5,000,000, in 26 below 3,000,000, and that the average of all on the first examination was 4,018,000. Of the 134 cases of this clinic, including those reported by Osler and McCrae, in 33 it was above 5,000,000, in 16 below 3,000,000, and the mean was about 4,000,000. The color-index is always considerably below unity unless the count be very low. Nucleated reds were rather rare. The count sometimes drops progressively till death (in one case to 1,786,000). The high counts are sometimes

attributable to the vomiting. The differential diagnosis between cancer of the stomach and pernicious anæmia is one of well-recognized difficulty, and in many cases can be settled only at autopsy. In general it may be said that in pernicious anæmia there is a lower count; that if the red blood-cells are below 1,500,000 it is against cancer; yet this rule is a broken reed, for it fails in both directions. Cases most like pernicious anæmia have small insignificant cancer nodules, and without autopsy would pass as primary anæmia. After autopsy one can by retrospect see minor points which should have led him to suspect cancer, but only then. In cancer the index is generally below one; there are fewer nucleated reds and those that are present are normoblasts as a rule; and leucocytosis is more common. In cancer the red blood-count is always higher than the cachexia would lead one to suppose, in pernicious anæmia the reverse. The blood examination Henry thinks of greater value in this differential diagnosis than the gastric analysis. The leucocytes in this disease vary so greatly and so without apparent explanation that little value can be placed on this count, except that there is a leucocytosis in over one-third of the cases, and in those without it the digestive leucocytosis is often absent (in 82 per cent. of 144 cases, yet this is of little really practical value, although it is one point to consider—Da Costa). Low counts, below 4000, are not rare. The highest of our series was 52,800.

It is said that the rapidity of growth controls the count, and yet our lowest counts included those certainly with metastases in liver, pancreas, or peritoneum (1600, 5400, 5000, 5600), and in 15 cases of cancer of the liver or abdominal organs generally the leucocytes were above 10,000 in but 7. In one case, 105,000 ( $t.^{\circ}$  103° F.); in another, 24,500 ( $t.^{\circ}$  99°.); just before death in a third, 61,400. The high counts were nearly all in those with the slight fever so often present.

Cabot reports a case with perforation into the peritoneum followed by quickly fatal peritonitis and a count of 105,600. We suspected this condition in a case the count of which rose to 120,000, an almost pure leucocytosis, but were unable to get an autopsy.

The percentage of large mononuclears has been found rather high (1 to 10 per cent.); in one case before death even 33 per cent. of a total count of 6300 (Kurpjeveit).

IN CARCINOMA OF THE ŒSOPHAGUS the blood is concentrated, giving a high content of dried substances; in v. Noorden's cases, 26.5 and 27.3 per cent. And yet in these cases there may be an oligæmia. If the cancer extends to the larynx, causing dyspnœa, the count may be high from the cyanosis.

Of our 6 recent cases, in one case the red count was 5,960,000, the lowest 4,184,000, and in another the first blood examination gave 4,696,000, 85 per cent. hæmoglobin, 6000 leucocytes; a later count was 6,476,000, 104, and 19,000 respectively. Five of the cases showed a leucocytosis, the highest 30,250. In 15 cases of cancer of the liver or general carcinosis of the abdominal organs, cases in which one might assume there was rapid and extensive growth, in but two was the red count below 4,000,000. In 27 cases of cancer of the bile-ducts

the lowest red count was 3,700,000, and in five the leucocytes above 10,000; in one case, 44,150 (t° 100° F.).

In 4 cases of CANCER OF THE RECTUM the reds were scarcely reduced (lowest, 3,732,000, the rest about normal). There was a leucocytosis in two cases [13,100 and 19,750 (t° 100° F.)].

In 10 cases of CANCER OF THE INTESTINE 3 showed marked anæmia; in one, reds 1,600,000; hæmoglobin, 40 per cent.; leucocytes, 2500; situation, the ileum: the other had 1,780,000 reds, 28 per cent. hæmoglobin, and 10,000 leucocytes; this patient was a nephritic as well: the third, reds, 1,609,000; hæmoglobin, 40; leucocytes (one week before), 7500; situation, the sigmoid flexure. The other red counts were from 4,000,000 to 4,500,000, four cases; the highest, 5,348,000; but 2 of the 9 showed a leucocytosis.

Our other cases of carcinomata showed no striking features, except one of the TESTICLE, with 2,832,000 red cells and 9600 leucocytes. Cancers of the kidney show usually high leucocyte counts, even 54,000. We had no such case. Also of the thyroid (71,000), and some of bone (52,700).

In sarcoma there is in general the same condition as in carcinoma, but some think that the effect on the blood is worse. We could not believe this from the study of our cases. This may be true particularly if the bone-marrow or the lymph-glands are especially involved; then a severe anæmia and high leucocytosis are the rule. In a case of osteosarcoma the red blood-cells were 663,400 (Hayem); in another case, 1,118,000; hæmoglobin, 28 per cent.; leucocytes, 68,200. In still another case, 2,240,000; hæmoglobin, 48 per cent.; and leucocytes, 54,000 (v. Limbeck's two cases). Yet other cases have counts even above 6,000,000, while still others present the appearance of a primary pernicious anæmia. The nucleated reds are said to be less numerous than in carcinoma. The hæmoglobin is said to be more reduced than in other cancers, the average being about 50 per cent., with 30 per cent. not rarely, while cases even below 10 per cent. are reported. The leucocytes in cases of osteosarcoma average about 17,000. They are more constantly increased and to a greater degree than in cancer, the cases resembling leukæmia. Among the qualitative changes an increase in the polymorphonuclear neutrophiles is less common than in cancer, but it may be present when there is no leucocytosis. This is said to have diagnostic value as against cancer. In some cases there is a large percentage of eosinophile cells, even 50 per cent., with little evidence that it is due to bone metastases. Myelocytes are sometimes increased. The old question whether all these cells are leucocytes or free sarcoma cells recurs often, for the increase often seems due to the small mononuclears.

**Lues.**—Lues, according to v. Limbeck, is the best illustration of

the dictum that no blood picture can be considered characteristic for the anæmia due to any one disease. In this case the blood picture may be the most varied, simulating anything from chlorosis to pernicious anæmia. The anæmia is particularly striking in the case of women. At first of the chlorotic type, it may at the end simulate a pernicious anæmia even of the severest grade, with a count of 428,000 cells; some cases of acquired lues have a practically normal blood, but this is unusual.

It is important not to confuse the anæmia due to the disease with that due to vigorous mercurial treatment. In a severe and protracted untreated case the chlorotic anæmia may reach an extreme grade, and then improve gradually.

During the *primary stage* a severe chlorotic anæmia is the rule, and any one following large European skin clinics is struck by the importance that is placed upon this anæmia, particularly in the case of women, in the diagnosis of a primary sore. Some say that the count will remain normal while the hæmoglobin diminishes considerably. During the transition from the primary to the secondary stage, one of the first signs of the dissemination of the disease throughout the whole body is the appearance of the rash and a further diminution of hæmoglobin. Yet the count remains nearly normal, while in a few days there may be a loss of hæmoglobin of 25 to 30 per cent. If untreated, the hæmoglobin soon may be as low as 25 per cent., and red blood-cells also drop, even at the rate of 230,000 cells per day. The severity of the anæmia depends on the condition of the patient, age, treatment, etc. In well-treated cases a rapid regeneration follows.

**LEUCOCYTES.**—During the tertiary stage with severe anæmia there is often a leucocytosis with a high lymphocytosis. Myelocytes are present in a severe case. This leucocytosis is an aid in excluding pernicious anæmia.

In an adult a high lymphocytosis and an eosinophilia suggest lues. In a child this blood picture might suggest rickets also. A low hæmoglobin per cent. and a high percentage of small mononuclears indicates a severe case.

In the primary stage the leucocytes are normal, or there is a slight leucocytosis with an increased percentage of lymphocytes. If mercury be given the percentage of the polymorphonuclear neutrophiles rises, the reverse of the action of mercury in a normal case.

During the secondaries the leucocytes vary from 12,000 to 16,000, the lymphocytes and eosinophiles are increased, the latter especially with the papular syphilide.

In 19 cases of secondary lues the red cells were diminished but slightly (minimum, 4,200,000; above 5,000,000 in 6), the hæmoglobin was evidently more affected

(40 to 90 per cent.; mean, 75 per cent.; color-index, 0.5 to 0.9; mean, 0.7), the leucocytes, as a rule, were normal (in 11 cases below 10,000, in 3 between 10,000 and 12,000), except in cases of high fever (leutic fever of secondary stage), of which there were 5 cases, in four with the leucocytes between 12,000 and 24,000, and dropping with the temperature.

There was a slight rise of leucocytes during the primary stage (average 9000). During the secondaries the count, depending on the skin lesion and the fever, varied from 9000 to 24,000, or even 50,000; average, 12,000 to 15,000. During the tertiary stage the counts varied greatly; in some cases there was a slight rise, in others a leucopenia. In hereditary lues the count has been found high,—12,000 to 24,000.

In hereditary and tertiary lues the red cells are seriously affected, in number, size and color. Megaloblasts are common. The blood picture, especially of the long-standing cases with much scarring of the organs and sclerotic bone-marrow may resemble primary pernicious anæmia. But in these conditions, as in cancer, the megalo-cytes do not predominate as they do in pernicious anæmia. Many cases in children reported as leutic anæmia were probably anæmia pseudoleukæmia infantum. Miller reported a case with 720,000 reds, 18 per cent. hæmoglobin, with normoblasts, megaloblasts, even gigantoblasts, microcytes, and poikilocytes. In the hereditary lues of infancy the anæmia may be fatal. The average leucocyte count of 25 cases was 7050. Large nucleated reds containing little hæmoglobin are important (Cima).

Following mild mercurial treatment the cells rise even 100,000 cells a day, the rise sometimes ending in even a slight hypercythæmia. But the rise is often preceded by a drop (since those red cells injured by the disease are first destroyed by the mercury?), hence sometimes a hæmoglobinuria and a drop in the count immediately after the inunction, followed by rapid regeneration and improvement in the general condition. If the treatment is carried too far it may itself be the cause of an anæmia. The length of treatment is therefore set at twenty-four days by some (Gaillard), but the individual variations are considerable. Conried advised from twenty-five to thirty-five inunctions; others say to give these *ad libitum*. In general the count rises for fourteen days, and if the mercury is continued begins to fall in twenty-four days.

Of 23 of our cases, 7 of which were of cerebral lues, the red cells were above 5,000,000 in 9 cases, between 4,000,000 and 5,000,000 in 10, and the lowest count 2,870,000. The color-index varied from 0.4 to 0.9; mean, 0.67. The leucocytes were below 10,000 in 20 of 29 cases. They varied from 10,000 to 18,500 in the other 9 cases; 6 were cases of high leutic fever; one was the malignant type (16,800, no fever); the case with 18,500 had large gummata. In one cerebral case the leucocytes were 3000, in another 2100.

**JUSTUS'S TEST.**—A large inunction or injection of mercury given

a case of lues before the rash, yet at a time when the general enlargement of the lymph-glands shows that the toxine is now disseminated throughout the whole body, is followed by an immediate drop in the hæmoglobin of from 10 to 20 per cent., and then a rise to normal or even above normal, in from one to several days, with improvement of all the symptoms. This drop, which is both rapid and considerable, is specific for a case of florid lues (but Cabot found it in a case of chlorosis). It may be obtained in any form of lues, late primary, secondary, tertiary, or hereditary, provided the disease be at that time florid, but not when or just before the symptoms begin to recede, as with cicatrization, desquamation, etc. It may again be obtained in case of relapse and until it has passed its climax. It is not present during the primary stage, so long as the infection is limited to the chancre and its neighboring glands, but only after the toxine has become generally spread, as shown by the swelling of distant glands. It cannot be used early to differentiate a hard and a soft chancre. This will explain the dissatisfaction many have with the Justus test.

As explanation it is thought that the mercury kills off the damaged red blood-cells which are soon replaced by new ones.

This test which promised so much has been repeatedly tried by various men. Many fail to get it in cases which turn out to be lues, yet in nearly all of these cases the test may have been applied too soon. Others have found the test in other diseases; for instance, Brown and Dale, Jones, Da Costa, Cabot, and Huger. Justus has lately reiterated his claim for its specificity,<sup>95</sup> and certainly answers well his critics. While the test may not be pathognomonic, it is still valuable.

**Renal Disease.**—The kidneys play an important part in the control of the composition of the blood, hence in nephritis the plasma changes are early and most important: a loss of albumin, lowered specific gravity, and in general all the signs of a chronic secondary anæmia. This may in part be due to the actual loss of albumin in the urine, the “serous hemorrhage” of some writers, but in acute cases, perhaps chronic, there is evidence of the presence of a toxine.

In the ACUTE HEMORRHAGIC NEPHRITIS especially the count may be very low, even 1,000,000, but usually the anæmia is moderate, and of this much is only apparent.

In 12 recent cases of ACUTE NEPHRITIS there were but two low counts, 2,600,000 and 2,900,000; a leucocytosis in five, 11,400 to 18,900. In Cabot's 50 cases, the lowest red count was 3,568,000, but the leucocytes were above normal in 31 of the cases; highest count, 50,000. Cabot thinks the leucocytosis due to hæmaturia or uræmia. But nephritis is an acute febrile, and perhaps infectious disease, and a leucocytosis is to be expected.

<sup>95</sup> Deutsches Arch. f. klin. Med., 1903, vol. lxxv. p. 1.

In CHRONIC NEPHRITIS so many factors come into play that the blood picture is not clear. Yet the influence of nearly all these factors is to cause anæmia. Among them is the condition of the heart; the œdema and hydræmia; the wretched condition of the gastro-intestinal tract, vomiting, diarrhœa, poor appetite, and the influence of the purges. The result is often a lowered count, a still more lowered hæmoglobin, and an hydræmic plasma.

In 103 cases of chronic nephritis the red cells were 1,700,000 in one case, between 2,000,000 and 3,000,000 in 13 cases, between 3,000,000 and 4,000,000 in 25, over 5,000,000 in 19; mean, 4,500,000.

The hæmoglobin in 99 cases was between 20 and 30 per cent. in 3 cases, from 30 to 50 in 29, above 80 per cent. in 17; mean, 62 per cent.; hence the mean color-index, 0.7, which is almost normal.

The leucocytes in 80 cases without uræmia were below 5000 in 4 cases, 5000 to 10,000 in 43, above 10,000 in 33, and of these the highest were between 20,000 and 30,000.

In 33 cases with uræmia the highest was 25,900, above 10,000 in 15 cases, below 5000 in 2; mean, about 9000. It is seen that in our cases those with uræmia did not differ much from those without.

A most interesting group is of the cases somewhat resembling pernicious anæmia, and with only nephritis to explain the condition.

A case from this clinic<sup>96</sup> is a good illustration of this, or of the simultaneous occurrence of the two diseases. The patient was a man 39 years old; red cells, 1,400,000; hæmoglobin, 27 per cent.; leucocytes, 7000 (pmn. n., 88 per cent.; s. m., 8 per cent.; l. m., 2 per cent.; eosinophiles, 2 per cent.). There was no poikilocytosis, and but one nucleated red found. The urine was of low specific gravity, with much albumin and many casts.

Cabot reports such a case with 1,468,000 reds; hæmoglobin, 23 per cent.; leucocytes, 3800 (pmn. n., 70 per cent.; s. m., 23 per cent.; l. m., 4.4 per cent.; eosinophiles, 2.6 per cent.; megaloblasts, normoblasts, and poikilocytes).

In the case of Labbé the red blood-count was 500,000; hæmoglobin, 2 gms.; the cells pale, irregular in form and size; nucleated reds rather small; mononuclears, 59 per cent. Recovery was rapid. He suggests that the anæmia was for the most part that of dilution. In another case the red blood-cells were 418,500 and the color-index over 1; and in a third the count was 1,000,000. At autopsy in such cases nephritis is the only lesion found. There were practically no signs of blood destruction, nor of regeneration, nor of megaloblastic degeneration of the marrow. It is a question how much of the low count the hydræmia will explain, but there is certainly some relation between the anæmia and the œdema, and the hydræmia which accompanies the anæmia.

We mention two other cases with arteriosclerosis and chronic nephritis. One was a woman, fifty-four years of age, with reds, 2,800,000; hæmoglobin, 50 per cent.; leucocytes, 6000; no fever. The other was a man thirty-two years old, with reds, 1,772,000; hæmoglobin, 22 per cent.; leucocytes, 50,000 (of which 91 per cent. were pmn. n.); the leucocytes later rose to 116,000; he left the hospital unimproved.

In interstitial nephritis the count is normal at first, and sometimes to the end. The condition of the heart is important. During the acute exacerbations, however, a slight lowering of the count is common, perhaps due to the hydræmia.

<sup>96</sup> McCrae, Johns Hopkins Hosp. Bull., October, 1902, p. 245.



In BILATERAL CYSTIC KIDNEY there was anæmia in both of 2 cases, 4,200,000 and 2,800,000; a leucocytosis of 13,500 and 36,000.

**Diseases of the Liver. CATARRHAL JAUNDICE.**—"Occasionally there is a slight leucocytosis at the onset, otherwise normal blood, with some degenerative changes in severe cases" (Cabot). An increased resistance, rigidity, and size is claimed for the red cells.

Of 27 of our cases, the red count was normal or even above normal in 16; the lowest, 3,000,000; the mean, 5,000,000 in the male patients. An interesting feature was the rise in the count of 300,000 to 750,000 cells while in the hospital. Of the 27 cases, in 20 the leucocyte count was 10,000 or below; in 3, from 10,200 to 10,500; and in 4 from 14,200 to 19,500; these cases all with slight temperature. The cells fell rapidly to normal after admission. A leucopenia follows in some cases (Bezancon and Labbé).

The plasma of the centrifugalized blood is bile-stained. Coagulation time slow.

**TOXIC JAUNDICE.**—Of this we had three fatal cases. Of one, the red cells were 3,570,000; hæmoglobin, 65 per cent.; leucocytes, 11,400: the second, 5,280,000, 75 per cent., 7000: and the third, 5,400,000, 65 per cent., and 12,500 respectively.

**GALL-STONES.**—A mild leucocytosis during the attack of colic is very common, a high one rare. During the colic the count in our cases (36 in number) took a sudden jump to about 15,000, but in cases of stone in the common duct with the chills and fever, it was higher, even 24,700. The red cells varied from 2,800,000 to 6,400,000; mean, 4,300,000. In a case with hemorrhage they fell to 1,880,000; hæmoglobin, 23 per cent.; leucocytes, 17,500. The coagulation time should be tested before any proposed operation, and if found lengthened calcium lactate may be given until it is normal.

**CHOLECYSTITIS.**—The leucocytes are invariably high, from 20,000 to 27,000 (Bloodgood). In one of our cases the count was 46,500. As the case becomes chronic the count falls nearly or quite to normal.

**CHOLANGITIS.**—Of 5 cases the leucocytes were 16,000, 33,160 (fatal), 15,600 (t.° 103.5°), 9000 (t.° 103°), and 6,400 (t.° 106°—fatal).

**ABSCESS OF LIVER.**—During the acute process the leucocytes may be high, but later are lower, or normal when the temperature is normal. Fletcher<sup>97</sup> found the average in 15 cases to be 18,350, the maximum 53,000. The reds were 2,600,000 to 5,600,000, mean, 4,200,000; mean of hæmoglobin, 60 per cent.

**CIRRHOSIS OF THE LIVER (ATROPHIC).**—Early there is no change in the red cells, later an anæmia. Da Costa's average, 3,404,000; Cabot's, 3,580,000; and one case, 1,300,000. The leucocytes are nor-

In our 32 cases the red cells varied from 3,100,000 to

<sup>97</sup> Jour. Am. Med. Assoc., August 22, 1903.



5,900,000, mean, 4,500,000; hæmoglobin mean, 68 per cent. Leucocytes in 30 per cent. of cases, over 10,000; the highest, 16,000.

**HYPERTROPHIC CIRRHOSIS (OF HANOT).**—Hayem reported a case with extreme anæmia. We have had five cases; in 2 the count was high, 7,800,000 and 8,500,000; and in one as low as Hayem's case, 1,504,000; hæmoglobin, 28; leucocytes, 6100 (the count rose later). In two there was leucocytosis (11,000 and 12,800).

**ACUTE YELLOW ATROPHY.**—The cases reported have had normal red blood-counts and moderate leucocytosis. In a recent case of this clinic, a boy 14 years old, the reds were 4,800,000 and leucocytes 12,700.

**Leprosy** (v. Limbeck).—Early no change is noted, but after a few years develops usually a pseudochlorosis with a normal count. After general malnutrition begins the anæmia becomes more marked, and yet is rarely very severe (in one case, however, 2,290,000 red blood-cells, 55 per cent. of hæmoglobin). Leucocytosis has not been found, these cells varying from 4000 to 8000 per cmm.

**Heart Disease.**—While compensation is good the blood is normal, but with acute loss of compensation and a low blood-pressure the blood is hydræmic, hence the count is low. With, however, the chronic stasis which follows, and the cyanosis, the blood-count rises and may conceal an anæmia. The worst anæmia is seen in aortic valvular insufficiency, as in a case with reds, 3,400,000; hæmoglobin, 30; leucocytes, 8000; and if the blood condition improves the heart may regain its compensation. In congenital heart disease with extreme cyanosis the condition of the blood is particularly interesting, as it is in these cases that we get not rarely a polycythæmia with red cells between eight and nine millions.

During the loss of compensation in 29 males with *pure mitral disease* the count varied from 3,000,000 to 7,500,000, and the mean 6,200,000. In 46 women the mean was 4,700,000, but the extremes, 3,500,000 and 8,000,000. There were interesting jumps of from 1,000,000 to 2,000,000 cells while under treatment.

In 37 cases of *pure aortic disease* the mean was 5,200,000; these cases showed a lower blood-count, as a rule, on each admission.

In 29 cases of *arteriosclerosis* (no important cardiac lesions) the mean was also 5,200,000. In 34 cases of *aneurism of the thoracic aorta* the mean was 5,500,000; in five men with aneurism of the abdominal aorta, 4,500,000.

**Addison's Disease.**—A hypocythæmia is the rule, the cells varying from 2,000,000 to 3,000,000; in one case 1,120,000; in other cases, however, the reverse is true, and the count may be even above 7,000,000. Some consider that the anæmia is due to a complication, and not to the disease itself. Others, that there is always an oligæmia,

but that this is covered by the concentration of the blood, and cite a case with true oligæmia and a count of 4,774,000 cells.

**Myxœdema.**—In myxœdema the count may be increased, diminished or normal. Many find an anæmia which improves with treatment. Some have found an increased diameter of the red blood-cells, which decreases under treatment, also many nucleated reds; that is, an infantile condition of the blood. The platelets were in a recent case much increased.

**Rickets.**—Anæmia is the rule, generally of a mild grade, but sometimes intense, rapid, and even pernicious.

**Scurvy.**—The count varies generally from about 3,000,000 to 4,000,000 cells. If the case is accompanied by much hemorrhage the anæmia is more intense. In Buchard's case, after three weeks with considerable epistaxis, the count was 557,000. In some grave cases macrocytes, microcytes, and fragmented reds are found. The color-index is reported low.

**The Value of Blood Examination.**—A question much discussed recently is the "value" of blood examination. But by "value" is usually meant the "practical use" which may be made of it, and not any interesting yet "useless" information it may throw on the case. The question is a fair one, especially since vast numbers of pages have been printed to prove blood counting indispensable.

The first point we wish to emphasize is that blood examination is of much greater value to the medical man than to the surgeon. The internist cannot dispense with it; the surgeon can. Some diseases are best diagnosed in this way. Among them are malaria; especially the forms without definite paroxysms and with atypical course, which pass otherwise as typhoid fever, meningitis, uræmic coma, pernicious anæmia, appendicitis, tuberculosis, dysentery, even Raynaud's disease (cases with superficial gangrene), the long list of diseases which atypical pernicious malaria may simulate, and failure to recognize which results in the unnecessary death of patient. Trypanosomiasis and infections with the Leishman-Donovan bodies can be recognized only by splenic puncture. Pernicious anæmia is quite uniformly overlooked without blood examination, and the cursory glance at a fresh blood specimen sometimes saves the patient from a course of treatment for jaundice, peripheral neuritis, or tabes, with which diagnosis patients are repeatedly admitted here. Blood examination is necessary for the diagnosis of splenomyelogenous leukæmia, and that this has a practical value is shown by the fact that the majority of our cases come to the surgical side for abdominal tumors (enlarged spleen), and are sent to us after a glance at the fresh blood. The diagnosis of lymphatic leukæmia, acute leukæmia, or pseudo-leukæmia, can be made only in this way.

For the early diagnosis of typhoid fever, measles, scarlet fever, etc., the leucocyte count is valuable, the absence of leucocytosis being much more suggestive than its presence; also in acute epidemic cerebrospinal meningitis, and various abscess formations, as of the liver or brain.

The leucocytosis is very valuable in pneumonia, especially central, and that of children and drunkards. An ever-increasing number of cases of trichinosis are recognized by the eosinophilia alone; chronic poisoning with coal-tar products was recognized in a neighboring city, notwithstanding the violent denials of the patient and her husband; various tuberculous infections are thus differentiated; the secondary anæmias due to cancer, from primary anæmia. These are only a few illustrations of the more interesting uses of blood examination.

For the surgeon, except as a differentiating diagnostician and for that he needs the blood report as much as the internist, the case is different. For him blood examination is almost synonymous with leucocyte counting. We can well appreciate the position of those men to whom blood-work is a novelty, who were successful surgeons before its day, who pride themselves that they do not need it and, indeed, are better off without it; of the men who try to use it, have never studied it themselves but must depend on assistants to interpret results for them, and who complain of the times they have been deceived; they expect too much from it; and of those who were once the blood-counting assistants themselves, who believe and often make good their boast that they can guess from the patient's general condition what the count in his case is, and if another figure is reported demand its confirmation or are sceptical as to the assistant's skill.

The question the surgeon usually demands of a leucocyte count is "Should I operate or not?" on a suspicious case of appendicitis, typhoid perforation, etc., and for this problem the count is half a point and the interpretation of it the other half. Almost never can the count decide the question alone. Some surgeons state they value it; more disregard it. The former never consider it as more than one of many symptoms, and very seldom as important an one as is the history, the physical examination, temperature, pulse, etc., but still of some help.

In acute abdominal and pelvic cases, when the question is one of an immediate operation, leucocyte counts are not indispensable. A man who knows well the field uses the blood report when convenient, that is all. One confession of its inadequacy is the value claimed for the iodine reaction, etc.

Both the medical man and the surgeon should remember that one count is seldom enough, any more than is one temperature determination enough; it is the series that counts in diagnosis, just as it is in

following a case awaiting operation. Unfortunately, blood examination takes time; yet not as much as is sometimes thought. A good hæmoglobin estimation can be made in from five to ten minutes, a leucocyte count in fifteen.

For our American clinics the message is, less routine blood-work, but a better quality of that which is done. The examination of the fresh specimen will save a great many unnecessary routine counts. But when the blood examination is important, as it so often is in internal medicine, the work should be well done and repeatedly done; well done as regards technic, consideration of the condition of the part pricked, the hour of the last large meal, etc.; repeatedly, until the curve is determined.

#### MALARIA

A few of the terms needing definition are the following: *Schizogone*, the asexual generation; *gametoschizont*, the sexual generation; *schizont*, or *monont*, a parasite of the asexual generation; *merozoite*, a segment (hyaline); *gamete form*, one of the sexual generation. Of the gamete forms, the *macrogamete* is the female cell; the *microgametocyte*, the parent male cell; and *microgamete* the male cell, which is one "flagellum" of the microgametocyte. *Sporogone*, the cycle in the mosquito; *vermiculus*, or *ookinet*, the motile fertilized macrogamete; *zygote*, *oöcyst*, *sporoblast*, are terms given to the spore cysts; *sporozoit*, the young sexual form which develops in the sporoblast, and which, when inoculated into the blood, becomes a hyaline.

By *pigment* is always meant the transformed hæmoglobin, or "melanin," the brown granules of which are seen in the fresh specimen, never the chromatin granules.

*Hyaline* always means a non-pigmented young form. A *ring form* is the shape which any young parasite may assume; it is not a "kind" of organism. *Presegmenters* are full-grown parasites the pigment of which has accumulated into masses and before segmentation appears.

The examination of the fresh blood is easy and satisfactory. The forms can be more easily recognized in this way than in the stained specimens. On the other hand, they are more easily found in stained specimens, and when very few the Ross method should be used. While a diagnosis may be made without blood examination in typical cases, it never will be made without it in certain atypical, even pernicious, cases without suggestive history or without fever, or with typhoidal temperature.

The "Tertian" Organism; *Hæmamoeba vivax* (Grassi); *Plasmodium vivax* (Plate IV).—This is the commonest form in Baltimore. Since the cycle extends over approximately forty-eight hours the paroxysms in the case of a single infection will occur on alternate days. The grouping is fairly definite, all the parasites undergoing their development quite in unison; the paroxysms occur during segmentation, and last from twelve to fourteen hours. In the case of a double infection there will be a paroxysm each day, "quotidian" fever, and in the blood will be seen two groups. Three groups very rarely occur, but we have seen one or two cases. The tertian hyalines (1-4) do not modify their

For the early diagnosis of typhoid fever, measles, scarlet fever, etc., the leucocyte count is valuable, the absence of leucocytosis being much more suggestive than its presence; also in acute epidemic cerebrospinal meningitis, and various abscess formations, as of the liver or brain.

The leucocytosis is very valuable in pneumonia, especially central, and that of children and drunkards. An ever-increasing number of cases of trichinosis are recognized by the eosinophilia alone; chronic poisoning with coal-tar products was recognized in a neighboring city, notwithstanding the violent denials of the patient and her husband; various tuberculous infections are thus differentiated; the secondary anæmias due to cancer, from primary anæmia. These are only a few illustrations of the more interesting uses of blood examination.

For the surgeon, except as a differentiating diagnostician and for that he needs the blood report as much as the internist, the case is different. For him blood examination is almost synonymous with leucocyte counting. We can well appreciate the position of those men to whom blood-work is a novelty, who were successful surgeons before its day, who pride themselves that they do not need it and, indeed, are better off without it; of the men who try to use it, have never studied it themselves but must depend on assistants to interpret results for them, and who complain of the times they have been deceived; they expect too much from it; and of those who were once the blood-counting assistants themselves, who believe and often make good their boast that they can guess from the patient's general condition what the count in his case is, and if another figure is reported demand its confirmation or are sceptical as to the assistant's skill.

The question the surgeon usually demands of a leucocyte count is "Should I operate or not?" on a suspicious case of appendicitis, typhoid perforation, etc., and for this problem the count is half a point and the interpretation of it the other half. Almost never can the count decide the question alone. Some surgeons state they value it; more disregard it. The former never consider it as more than one of many symptoms, and very seldom as important an one as is the history, the physical examination, temperature, pulse, etc., but still of some help.

In acute abdominal and pelvic cases, when the question is one of an immediate operation, leucocyte counts are not indispensable. A man who knows well the field uses the blood report when convenient, that is all. One confession of its inadequacy is the value claimed for the iodine reaction, etc.

Both the medical man and the surgeon should remember that one count is seldom enough, any more than is one temperature determination enough; it is the series that counts in diagnosis, just as it is in

### PLATE III.

#### THE BLOOD IN TERTIAN MALARIA.

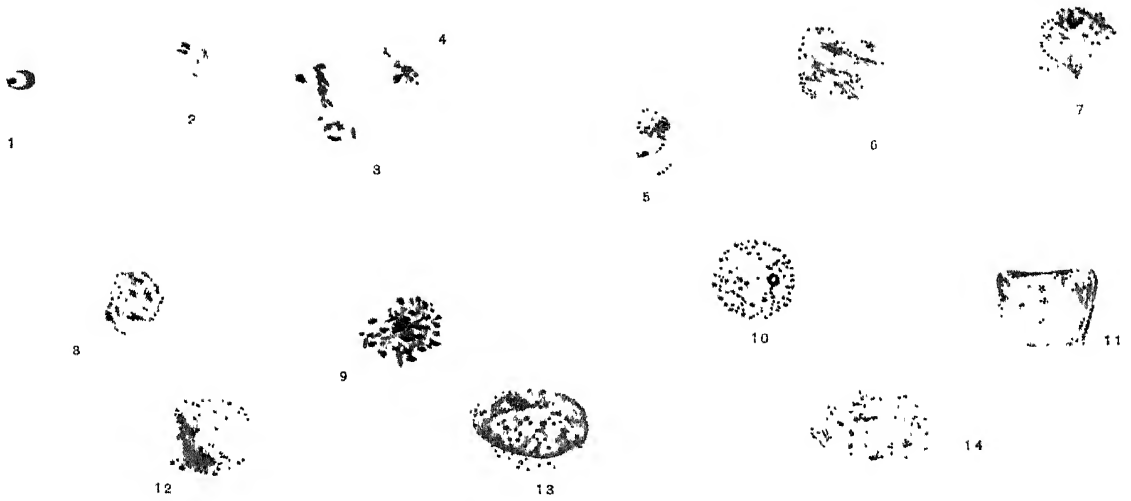
1. A hyaline form.
2. A young tertian, perhaps twelve hours old, with beginning granulation.
- 3, 4, 5, 6. Half grown, and slightly older, forms. 3 is a large cell containing two parasites.
7. A form almost full grown.
- 8, 9. Full grown parasites showing division of the chromatin preceding that of the cell.
10. A small tertian parasite in a red cell showing "stippling" (Plehn's granules).
- 11, 12, 13, 14. Gamete (sexual) forms. 13, in a cell with Plehn's granules.

#### THE BLOOD IN QUARTAN MALARIA.

15. A very young quartan parasite.
16. A full grown form with the chromatin still in one clump.
17. A full grown form with the chromatin scattered.
18. A segmenting parasite.

#### THE BLOOD IN AESTIVO-AUTUMNAL MALARIA.

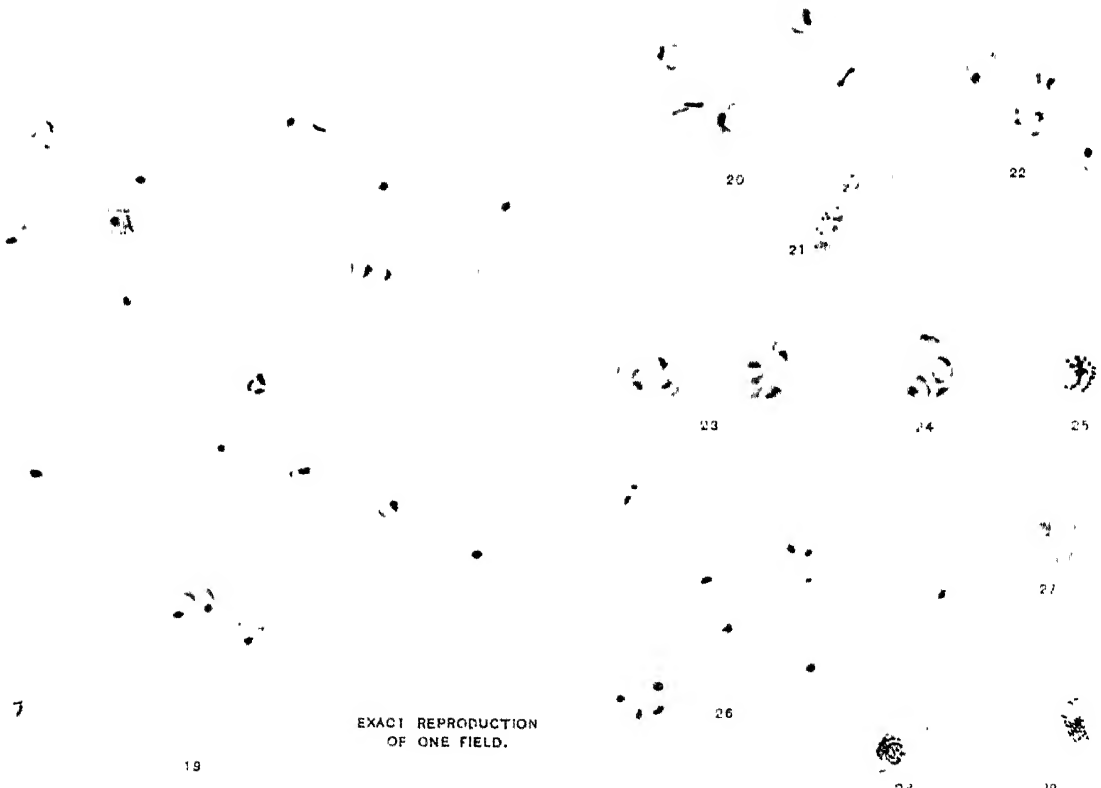
19. One field exactly reproduced from the blood of a case of pernicious malaria.
20. Aestivo-Autumnal hyalines showing the projection of the chromatin masses from the cells.
21. Blood platelets.
22. Aestivo-Autumnal hyalines.
- 23, 24. Red cells containing more than one hyaline.
25. A full grown aestivo-autumnal parasite.
26. Hyalines free in the plasma.
27. A blood platelet lying on a red corpuscle.
- 28, 29. Crescents.



TERTIAN MALARIA.



QUARTAN MALARIA.



EXACT REPRODUCTION  
OF ONE FIELD.

ÆSTIVO-AUTUMNAL MALARIA.

STAINED WITH HASTING'S MODIFICATION OF  
ROMANOWSKI'S STAIN. ALL DRAWN TO SAME SCALE.

*P. S. Lockwood*





red blood-cell host either in size, color, or contour. The parasite is small, a little over 2 microns in diameter, colorless, non-pigmented, often disk-shaped, with an undulating periphery. It makes very rapid amoeboid movements and produces an extraordinary series of changes of shape and position. It also assumes the typical ring form once supposed to be characteristic of the *æstivo-autumnal* parasite. This ring is usually a little thicker at one point, hence the name "signet ring." In one cell may be one, two, or even five, such forms. In about twelve hours the corpuscle (6-7) will be a little larger, a little paler, but with a sharp, smooth, round margin. The organism is exceedingly amoeboid, the pseudopods often many in number, and so thread-like and pale that their connections can scarcely be seen; hence the cell may seem to contain a number of disconnected globules of pigmented protoplasm. The protoplasm is so little refractive that the outline of the parasite is difficult to make out. (It is thought by some that the parasite is more distinct and sluggish after the patient has begun to take quinine.) The pigment has at this age appeared in moderate amount, and consists of very fine, light-brown granules, which dance with a motion so rapid that waves in the protoplasm must be assumed. The pigment is clustered especially at the ends of the pseudopods. The untrained eye, particularly of one who has not yet learned how to light the specimen well, will see merely a swollen pale corpuscle in which dance very fine pigment granules. At the end of twenty-four hours the cell (Plate IV, 8) is somewhat larger, paler, but still round in outline. The organism now fills about one-third of the cell. It is still quite amoeboid, but less actively so. The pigment has increased in amount, is a little darker, a little coarser, a little quieter, and is evenly distributed through the substance of the parasite. The nuclei of these forms can sometimes be seen in the fresh specimen as a globular body at the end of a pseudopod, and especially in the degenerated extracellulars when spread out against other cells.

During the last half of the cycle the growth is more rapid, and hence students often judge the age wrongly, considering size directly proportional to age. At forty hours the parasite (9) is full-grown. The cell is now about one and a half times the normal size. It is so pale that its outline will hardly be seen; is, in reality, nothing but a shadow. The organism is from 8 to 10 microns in diameter, is round, and so little refractive that it is practically impossible to say where the parasite leaves off and the corpuscle begins. The pigment is more abundant and is evenly distributed throughout the parasite, an important point in diagnosis, since in the *quartan* at this age it will be practically all in the periphery, and in the *æstivo-autumnal* at the centre.

The next stage is the "presegmenter." The corpuscle is now

almost or quite invisible. The pigment collects in one or more irregular clumps, the granules moving in irregular lines to form these masses. The organism is next a "segmenter" (10-17). The corpuscle is no longer seen, the organism is slightly more opaque, denser, more refractive. Refractive dots appear irregularly in the body of the protoplasm, from 15 to 20 in number, crenations are seen at the margin, and lines of separation appear around these refractive dots marking off the future segments. The segments now become more sharply defined, until finally we have a clump of fifteen or twenty discrete circular masses with a refractive dot in the centre. The clump may be irregular or form two quite concentric circles. The pigment is merely left in masses between these segments. The segmenter now seems to burst, and the young organisms spring apart. Each segment is a hyaline, and is ready for a new cell as host.

The whole cycle may occur in the peripheral blood, but the number of segmenters found will not be as large as would be supposed from the number of parasites seen a few hours previously, since so many of them have accumulated in the internal organs. A few hours after the first segment appears the chill begins.

The above is a description of a typical tertian parasite. One finds, however, some variations in this group. In one rare form, a few cases of which we meet with each year, the parasite forms more pigment than usual and in large coarse granules, but of a lighter brown color than those of the quartan or the adult æstivo-autumnal, and which form dense clusters at the ends of the pseudopods, so filling them that the granules cannot dance at all. The fine thread-like pseudopods stand out with great distinctness. The cell containing it is often not swollen but very pale, yet in one such case all the full-grown forms found were in cells from 8.5 to 13.3 microns in diameter.

Pigmented leucocytes are common (perhaps since the pigment granules are conspicuous).

The grouping does not seem to be so definite, and hence the chills are slightly longer than usual.

*Extracellular Tertian Forms.*—These are of two varieties, the degeneration forms and the gametocytes. The degeneration forms, or the extruded intracellulars (18-21), may in a short time after the specimen is made be the only ones seen. These are parasites which have burst from their cells and died. The organism is often seen to "run out" as if through a very fine hole. If it entirely escapes the hæmoglobin leaves the cell through the same opening, and only a shadow is left, but very often it does not, and hence we have the dumb-bell-shaped form with the constriction at the orifice. After the parasite is free in the plasma the pigment will for a time be extremely active in movement and then gradually become quiet, as the organism dies and then degenerates. It may break up into fragments, forming a string of four or five small pigmented spherical masses (20, 21), or

it may become deformed or swollen and vacuolated, the so-called "sporulating forms" which much resemble reproduction forms (23, 24).

The more interesting extracellulars (but stained specimens show that these are surrounded by the shell of a corpuscle) are the gametocytes, which correspond to the crescents of the æstivo-autumnal form, but have a less distinctive shape. Like them they can be found at all times in the blood after a few days of the infection. The macrogamete was formerly considered a cadaveric form, and was known as a "swollen extracellular." They are large organisms, pale and indistinct, some three or four times the size of a red blood-cell. In some no trace of a corpuscle can be seen, the pigment is abundant, in very coarse rods, and in very active movement. The nucleus is about 3.5 microns in diameter, and is often evident in the fresh specimen; either its outline can be seen, or its size and shape may be recognized since it is the only portion of the parasite which is not invaded by the pigment granules. The extreme vitality of these cells is astonishing, as might be expected from the fact that it is their function to continue the life of the organism in the mosquito. Recently one with particularly active granules was left by a student under his microscope in a moderately warm room. Eighteen hours later the pigment was still actively dancing. Whether these very large forms with such active pigment and quite unlike those in the stained specimens we call macrogamete forms, are the same or are fertilized forms, I do not know. The microgametocytes, smaller than the former, are from 8 to 10 microns in diameter. The pigment is in active motion, but soon forms a circle around the centre and becomes stationary. As a rule, nothing more happens. But the pigment, instead of collecting, may become even more active, as if stirred up by something moving within the cell. The margin may undulate, and the flagella, four or five in number, burst out. These flagella are the microgametes or male elements. Although the name "flagellum" is still used, it should be borne in mind that it is decidedly a misnomer. They are threads whose length is from two to three times the diameter of a red blood-cell. Sometimes these threads are rendered irregular by fusiform masses of protoplasm often containing pigment granules. These make them more conspicuous and much easier to follow when they break loose, and wander for more than an hour through the field. After the flagella have broken loose a small cell, with its pigment near the centre, is all that remains. This process of flagellation is not seen in the very fresh specimen, but occurs in from fifteen to twenty minutes after the blood has been drawn, proof that it does not occur in the body, but under the stimulus probably of the lowered temperature.

**Quartan Malaria.** *Hæmamoeba malarix* (Plate IV).—Of this rare form

we see but one or two cases a year. The cycle of development requires seventy-two hours, hence if but one group is present the paroxysms will occur on each fourth day; if two groups, there will be two days with paroxysms, and then one free day, followed by two more paroxysms; if three, quotidian fever, providing each group is large enough numerically to cause a chill. The grouping of this parasite is even more uniform than that of the tertian, the forms being nearer of the same age, and hence the paroxysms are slightly shorter, often requiring but ten hours.

The hyalines (26) cannot be distinguished from the tertian, but may be a little later when the pigment first appears (27), since the granules are coarser, blacker, and less actively vibratory. As the parasite grows the corpuscle becomes slightly smaller and shrunken, and with an irregular crenated margin; but much deformity is rare. The protoplasm of the parasite is more refractive than of the tertian, even looks waxy, hence the outline of the pseudopods is easily seen. The parasite is definitely amœboid, but not actively so.

In twenty-four hours the cell is smaller, crenated, and brassy in color. The organism is round or oval, sometimes slightly amœboid, but very sluggishly so. It is very distinct, since refractive. The pigment is coarse, blackish-brown in color, gathered at the periphery, especially on one side. At this age all motion of the pigment granules has practically ceased. The parasite soon fills from one-third to one-half of the cell (30, 31), becomes rounder, and is non-amœboid. The cell may be shrunken, crenated, and brassy, although some may not seem in the least altered. The pigment is coarse, much blacker than the tertian, motionless, and entirely at the periphery. The protoplasm is very distinct, refractive, and waxy in appearance.

During the third day only a rim of the cell is left, and this is usually of a dark, brassy color. The organism (32-34) is now full-grown and about 7 microns in diameter, the figure usually given.

We have measured a good many quartan parasites, and, contrary to this, find that of 135 full grown and segmenters, 60 per cent. were from 7.4 to 8.1 microns in diameter, and only 18 per cent. small, from 6.2 to 7 microns. Of those two-thirds grown, 43 per cent. were in cells from 6.2 to 7 microns in diameter, the rest in cells of normal size.

At sixty hours the cell can hardly be seen. The organism is round or elliptical and motionless (35). The coarse dark pigment is all at the periphery.

The pigment now flows to the centre in definite streams along radial channels, thus giving a beautiful wheel-like picture (36), the rows of pigment granules forming the spokes. The granules finally collect in a clump at the centre. These are the presegmenters. The seg-



## PLATE IV

THE PARASITE OF TERTIAN FEVER. (Drawn by Mr. Brödel for Thayer and Hewetson's paper, The Malarial Fevers of Baltimore, Johns Hopkins Hospital Reports, Volume V. We copy the original legend.)

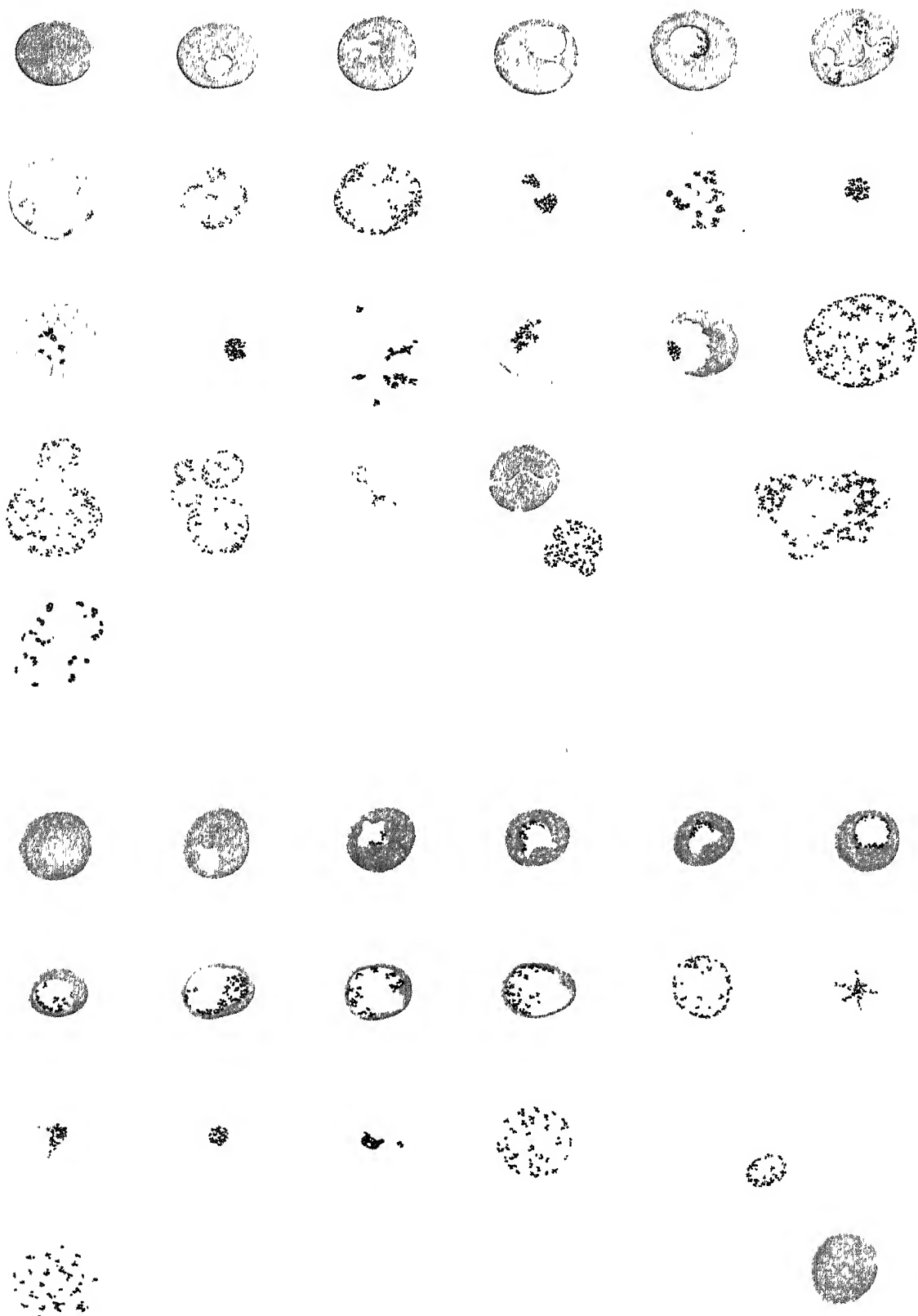
1. Normal red corpuscle.
- 2, 3, 4. Young hyaline forms. In 4, a corpuscle contains three distinct parasites.
- 5, 21. Beginning of pigmentation. The parasite was observed to form a true ring by the confluence of two pseudopodia. During observation the body burst from the corpuscle, which became decolorized and disappeared from view. The parasite became, almost immediately, deformed and motionless, as shown in Fig. 21.
- 6, 7, 8. Partly developed pigmented forms.
9. Full grown body.
- 10-14. Segmenting bodies.
15. Form simulating a segmenting body. The significance of these forms, several of which have been observed, is not clear to the writers, who have never met with similar bodies in stained specimens so as to be able to study the structure of the individual segments.
- 16, 17. Precocious segmentation.
- 18, 19, 20. Large swollen and fragmenting extra-cellular bodies.
22. Flagellate body.
- 23, 24. Vacuolization.

THE PARASITE OF QUARTAN FEVER.

25. Normal red corpuscle.
26. Young hyaline form.
- 27-34. Gradual development of the intra-corpuscular bodies.
35. Full grown body. The substance of the red corpuscle is no more visible in the fresh specimen.
- 36-39. Segmenting bodies.
40. Large swollen extra-cellular forms.
41. Flagellate body.
42. Vacuolization.

The Parasite of Tertian Fever

PLATE IV.







menters are among the most beautiful things seen under the microscope. The organism becomes opaque and very waxy; refractive dots appear in a single regular circle around the periphery; crenations of the border appear with these dots as their centre; lines of division start from these and run to the centre, forming from six to twelve rays like the petals of a flower, hence the names "daisy," "marguerite," or "rosette" form (37, 38). These segments then separate as in the tertian. The whole cycle of the quartan occurs in the peripheral blood, hence one finds about as many segmenters as the number of the full-grown parasites would lead one to expect.

The gamete forms (40, 41) are very seldom seen. They are similar but somewhat smaller than the tertian. Flagellation occurs in the same way. The extracellular degenerate forms are found, although the parasite keeps in the cell much better than does the tertian.

In review, the differences between the tertian and the quartan may be stated as follows: The cycle of the quartan is seventy-two instead of forty-eight hours. This organism is, throughout its entire history, smaller, more refractive, less amœboid, its pigment is coarser, blacker, less vibratory than the tertian, and keeps a peripheral position. The corpuscle is shrunken, crenated, and brassy. The presegmenter and segmenter forms of the quartan are perfectly distinctive, since they have such geometrically regular forms. The number of the segments is small, from 6 to 12; and, lastly, more of the segmenting forms are found in the peripheral blood.

**Æstivo-autumnal. Plasmodium præcox. Hæmatozoon falciparum** (Plate V).—This is a common form, particularly in the Tropics, and the most dangerous of the three. In the fresh infection the grouping is quite definite, but soon the members of a group lose their unison, and hence are found in the internal organs of all ages at once. For this reason what was first an intermittent fever becomes more and more continuous, until finally the temperature may resemble that of typhoid fever. The duration of the cycle is rather uncertain. Dr. Thayer considers that while usually of about forty-eight hours, it may vary from twenty-four to perhaps seventy-two.

All students, it is said, "pass through the stage" of dividing this form into "benign," "malignant," "pigmented," "non-pigmented," etc., varieties, but most recover, especially those who follow the splenic blood carefully. Some separate it into the "malignant quotidian" and the "malignant tertian," the latter similar in form to the "benign tertian" (ordinary tertian), except smaller, from one-third to one-half the size of red cells, and with from 8 to 12 segments. The "malignant quotidian" is from one-third to one-fifth the size of red cells, "often unpigmented" and with from 6 to 8 spores. In this locality we are often struck by the great difference in the æstivo-autumnal parasites, both clinically and morphologically, especially as regards the amount of pigment and the presence in the circulation of the adult much-pigmented forms, but Dr. Thayer thinks such division not justified as yet.

The hyalines are similar to those of the tertian and the quartan, perhaps are slightly smaller, but they assume the signet-ring form much more commonly and hold it longer. Then they are very refractive, hence easily seen, but may at any time lose this refractivity and become amoeboid exactly as does the tertian. In a severe infection even five rings may occupy one cell.

As the parasite (7-12) grows a very slight amount, usually but one or two granules, of pigment appears. These are so fine that they are easily overlooked. These are motionless as a rule, although sometimes slightly dancing, and are seen at the periphery of the parasite or at the inner edge of the biconcavity. The cell is commonly very much injured, shrunk, crenated, and brassy, even when the parasite is very young, and yet some infected cells look normal. A great many cells which contain no parasite also show these same evidences of being poisoned. The parasite at this stage fills about one-fifth of the cell. As a rule the infected cells now disappear from the peripheral circulation, perhaps since the injured cells are treated as foreign bodies, hence are filtered out, and to study their further development the spleen must be punctured. The suddenness of their departure is quite surprising, as well as exasperating to a demonstrator, since in two hours a large brood may disappear. Hence it is that if no crescents are present the diagnosis will be uncertain unless repeated examinations of the blood are made. In some cases, however, all ages of this parasite may be found in the peripheral blood. In these cases and in the blood obtained by splenic puncture, the pigment is seen to increase considerably in amount, and to be in rather coarse, dark granules, or remains scanty; while in some scarcely any seems to form. Those parasites with much coarse black pigment it is impossible to tell from quartan forms, and this mistake is frequently made. The more malignant the parasite the fewer older forms are seen in the peripheral blood, and according to some the less pigment is formed; the pernicious cases always have abundant young parasites in the peripheral blood. In some cases the hæmoglobin seems to gather around the parasite, leaving an almost colorless ring at the periphery of the red cell (13). In the internal organs the whole cycle seems to occur inside of large macrophages. The parasite grows to about one-half the size of the cell (5 microns). When full-grown the pigment is all in the centre (15-20), never diffusely scattered, and never peripheral. The protoplasm is waxy. This form is very characteristic. Although rarely seen in the circulation, in two cases recently during the class demonstration we found several beautiful full-grown and one segmenting form (see Fig. 93). The segmenters vary in size from 2.5 to 5 microns in diameter. The process of segmentation (21-24) is similar to that of the tertian, the waxy



## PLATE V

THE PARASITE OF AESTIVO-AUTUMNAL FEVER. (Drawn by Mr. Brödel for Thayer and Hewetson's paper, The Malarial Fevers of Baltimore, Johns Hopkins Hospital Reports, Vol. V. We copy the original legend.)

- 1, 2. Small refractive ring-like bodies.
- 3-6. Larger disc-like and amœboid forms.
7. Ring-like body with a few pigment granules in a brassy, shrunken corpuscle.
- 8, 9, 10, 12. Similar pigmented bodies.
11. Amœboid body with pigment.
13. Body with a central clump of pigment in a corpuscle, showing a retraction of the hæmoglobin-containing substance about the parasite.
- 14-20. Larger bodies with central pigment clumps or blocks.
- 21-24. Segmenting bodies from the spleen. Figs. 21-23 represent one body where the entire process of segmentation was observed. The segments, eighteen in number, were accurately counted before separation as in Fig. 23. The sudden separation of the segments, occurring as though some retaining membrane were ruptured, was observed.
- 25-33. Crescents and ovoid bodies. Figs. 30 and 31 represent one body which was seen to extrude slowly and, later, to withdraw two rounded protrusions.
- 34, 35. Round bodies.
36. "Gemmation," fragmentation.
37. Vacuolization of a crescent.
- 38-40. Flagellation. The figures represent one organism. The blood was taken from the ear at 4.15 p.m.; at 4.17 the body was as represented in Fig. 38. At 4.27 the flagella appeared; at 4.33 two of the flagella had already broken away from the mother body.
- 41-45. Phagocytosis. Traced by Dr. Oppenheimer with the camera lucida.

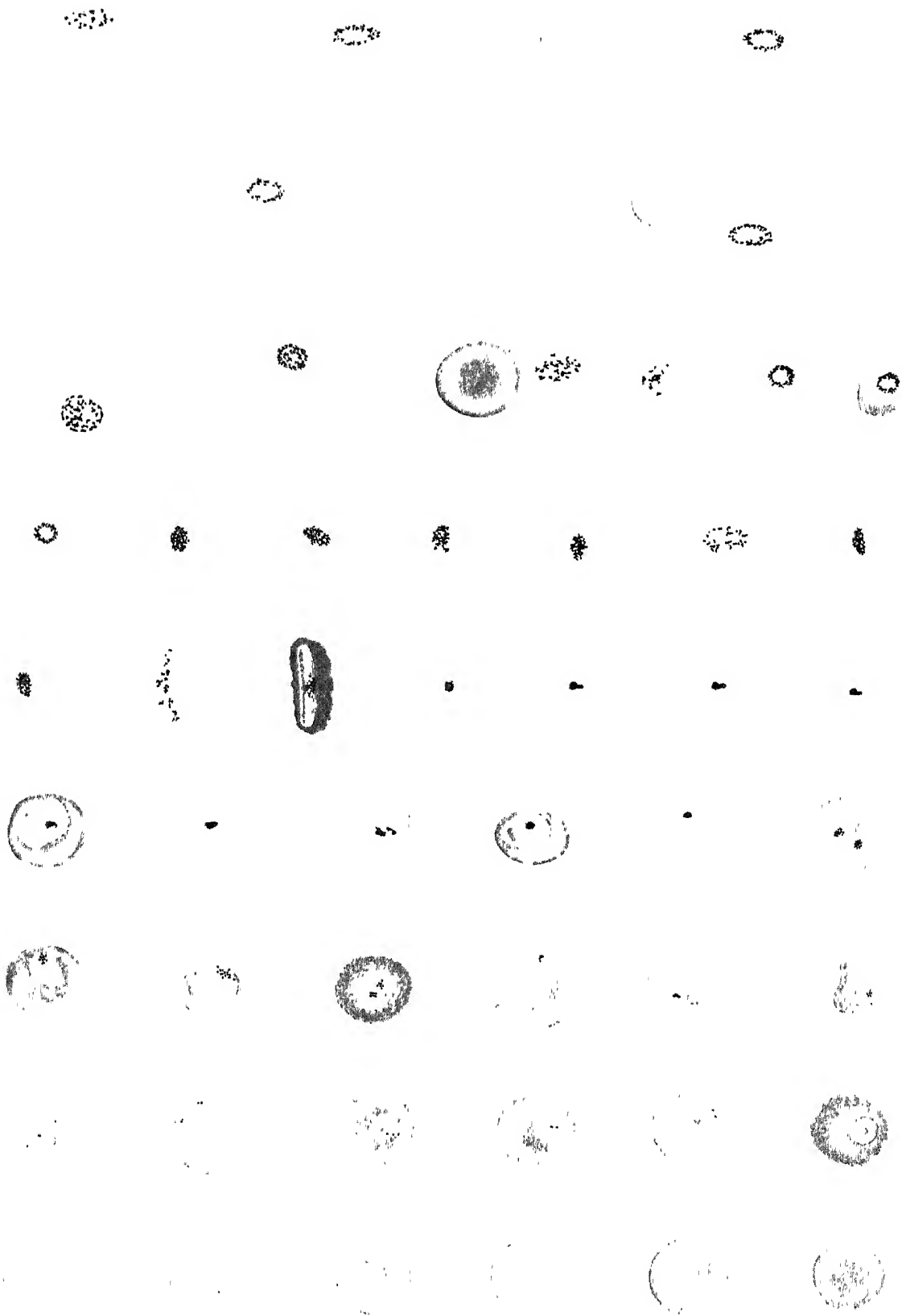


PLATE V.



opaque organism breaking up irregularly into fifteen or sixteen very small segments. Very few degenerated extracellulars are found.

*Crescents and Ovoids.*—These very characteristic forms of the æstivo-autumnal parasite are found in the internal organs from about the fifth day of a fresh infection, and appear in the peripheral blood on about the seventh day. The crescents (29) are slightly longer than the red blood-cells, sometimes of a beautiful crescentic shape with rounded ends; others are somewhat irregular. They are very refractive, with a double contour, and usually present a fringe of the degenerated red blood-cell which in the concavity is somewhat more abundant and forms the so-called “bib.” The pigment is considerable in amount, clustered at the centre of the crescent either as a confused mass, a sheaf, or a ring. The granules are coarse and usually rod-shaped. While watching the parasite it may lose its crescentic shape and become first oval (ovoids, 30–33), then circular (34–36), or it may resume the crescentic shape. Around the circular there is no trace of the corpuscle left; its protoplasm is much less refractive than the crescent. Two forms of the circulars have been described in the fresh blood, the macrogamete and the microgametocyte (see page 636). The former may flagellate. Fertilization has been watched by several observers, first of all by MacCallum<sup>98</sup> and then by others of this hospital, and more recently by Moore, *et al.*<sup>99</sup> Vacuolation and fragmentation of these sexual forms are not rare (Plate V, 37).

The phagocytes are well studied in this form of malaria; in fact, pigmented leucocytes are as valuable in diagnosis as is the parasite itself. These are large mononuclears especially, polymorphonuclear neutrophils, and macrophages (see page 621, and Fig. 93). Pigmented macrophages are seen only in severe cases. In these phagocytic cells are found free pigment granules, or masses of pigment, or parasites, especially segmenters and flagellates. In the tertian and quartan they occur just after a chill, but in the æstivo-autumnal at any time. The large macrophages especially contain organisms, even those within cells. Some of these macrophages are necrotic.

The malarial pigment is black, “melanin,” and iron cannot be demonstrated in it.

**The Cycle within the Mosquito.**—This cycle has been followed by several observers in the case of *Plasmodium præcox*. The crescents in the blood in the stomach of the mosquito become circular forms. The male circulars flagellate in the same way as on the stage of the microscope, and probably in response to the same stimulus, the lowering of temperature. The flagella then fertilize the female circulars. This occurs in from 1 to 1.5 hours after the mosquito has bitten. During the flagellation of the microgametocyte the macrogamete ripens by casting off karyosomes, polar bodies consisting of chromatin, and projects a

<sup>98</sup> Johns Hopkins Hosp. Bull., November, 1897.

<sup>99</sup> Johns Hopkins Hosp. Bull., October, 1902.

slight mound, through which the free flagellum has been seen to enter. The nuclear material of the macrogamete and the microgamete then unite. The cell remains naked and assumes a motile spindle form called the "vermiculus." Its size varies from 20 microns up, and is to be found in from forty to forty-eight hours after the blood has been ingested. This motile form in the case of malaria has been found only in the contents of the mosquito's stomach. This vermiculus

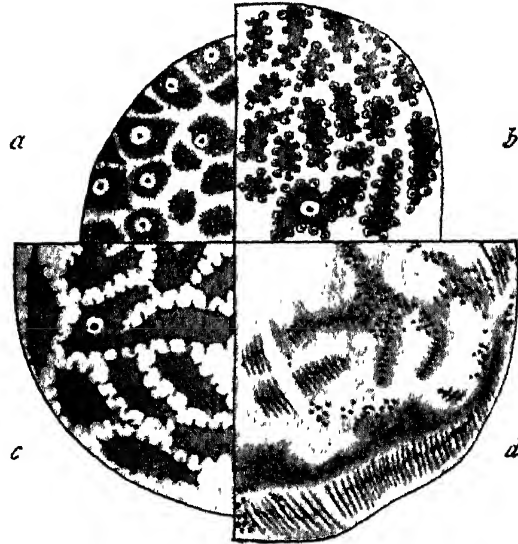


FIG. 116.—Various stages of the development of *Plasmodium praecox* in the mosquito's stomach. *a*, In four to four and a half days after the bite; *b*, *c*, five to six days; *d*, eight days (*Plasmodium vivax*). (From Braun.)

actively bores its way through the epithelial cells of the intestinal wall, and becomes encysted between the intestinal epithelium and the elastic layer, the "tunica elastico-muscularis," which forms the membrane of the oöcyst. This oöcyst now increases in size. The nucleus divides rapidly. As the cyst grows it bulges outward from the intestinal wall, forming a pendulous tumor into the body cavity (see Fig. 117). These vary from 4.5 to 30, or even 60, and as high as 90 microns in diameter. This

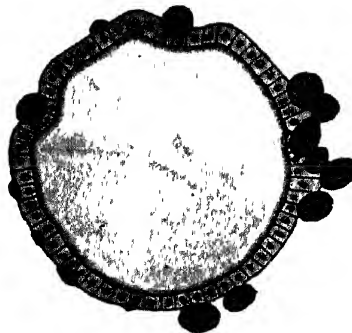


FIG. 117.—The intestine of an infected mosquito with oöcysts attached. (From Braun.)

stage is called the "medium zygote," or the "medium sporoblast," and is conspicuous because of the amount of pigment. There may be 200 such tumors attached to the intestine of the mosquito. The protoplasm now gathers around the divided nuclei (Fig. 116, *a*), a process analogous to the sporoblast formation of the coccidia except that here the separation is less perfect, the daughter cysts being connected by bridges of protoplasm. It is now known as a "large zygote"



or a "large sporoblast." In each of these divisions the nucleus divides into great numbers (*b*, *c*), the daughter nuclei remaining on the surface of the various daughter cysts. The protoplasm collects around each, first forming spherical cells, which then elongate into threads lying parallel in masses over the residue of the sporoblasts. These threads are called "sporozoites." Their nucleus also becomes elongated. The final length of these sporozoites is about 14 microns, and the width about 1 micron. Their protoplasm is thick, homogeneous, and very refractive. All sporozoites of one oöcyst ripen at about the same time; they may be present even to the number of 10,000 in some oöcysts, while others contain but a few hundreds. When ripe the oöcyst bursts into the body cavity, the sporozoites wander free, but, as if directed by some positive chemotactic influence, finally collect in the salivary glands. They are motile, moving by a bending gliding movement. Inoculated by the mosquito's bite into the blood-vessel of man, they attach themselves to and finally penetrate into the red blood-cells, a process actually observed by Schaudinn. They are said to stay for some time on the surface of the cell before penetrating, and it is said that if quinine is now given they will drop off from the corpuscle. This may explain the opinion of several recent writers who insist that the hyaline and older forms are always on and not within the corpuscle. As a rule the first chill will come on about the eighth or twelfth day after the mosquito bite, although, of course, it will depend on the number and the

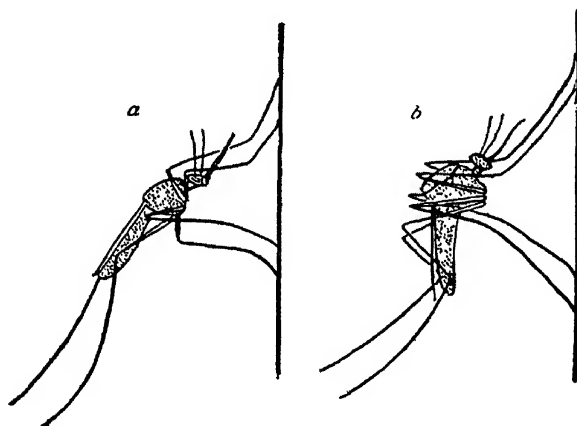


FIG. 118.—Attitude of mosquitoes on wall. *a*, *Anopheles*; *b*, *Culex*.

virulence of the parasites introduced into the circulation. Since some mosquitoes contain fully 200 of these oöcysts (of course not all of the same age), and some of these contain 10,000 or more sporozoites, the number of the hyalines injected by one bite may be considerable.

For the tertian the optimum temperature for this cycle is 28° to 30° C., and the time eight days; below 17° to 20° C. there is no development. The quartan form can develop at a slightly lower temperature.

The *Anopheles* group of mosquitoes is the only one as yet shown to be the host of the malarial organism. For a full description of these insects the reader is referred to various books.<sup>100</sup>

The *Anopheles* genus may easily be recognized by its attitude on a wall, since (see Fig. 118, *a*) its body is in a straight line with head and proboscis, and at an angle with the wall, the "awl shape," while *Culex* (*b*) sits "hunch-backed," its body parallel to the wall, its proboscis at an angle of forty-five degrees with its body. The genera are separated by the relative length of their probosces and palpi (see Fig. 119). Of the *Anopheles* female these are of equal length and scaled, while of the *Culex*, *Stegomyia*, and *Tæmiorhyncus* females the palpi are short and insignificant. It is only the female *Anopheles* which bites. The wings

<sup>100</sup> Stevens and Christophers, "Malaria of the Tropics," 1905; Nuttall and Shipley, *Jour. of Hygiene*, vol. i., Nos. 1 and 4; vol. iii., No. 2.

of *Anopheles* alone are spotted, as a rule. *Anopheles* usually holds its hind pair of legs stretched out and oscillating in the air.

Its egg and larva are characteristic: the former, from its boat-like shape and lateral air-cell floats; the latter, from its attitude in the water, lying parallel with and just below the surface.

*Stained Specimens.*—The technique is fairly simple. Very thin smears must be made (see page 474); these are stained by any one of the various polychrome methylene blue-eosin mixtures (see page 481). The fresher the smear when stained the better the preparation.

Ross has recently described a method which is of the greatest value when only a few parasites are present. A very thick drop of blood is placed on the slide and spread over an area equal in size to a ten-cent piece. It is then dried thoroughly in the air. The slide is then covered with water in order to remove the hæmoglobin. Care should be taken that the washing be not too vigorous, else the fresh

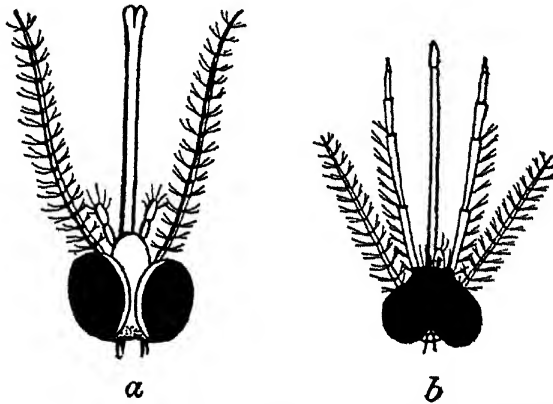


FIG. 119.—Heads of mosquitoes. *a*, *Culex*; *b*, *Anopheles*.

blood which has not been fixed will be washed off. The specimen is then stained in the usual manner. In such a specimen parasites appear numerous, when in ordinary smears scarcely one is found.

**TERTIAN** (Plate III, 1–14).—The youngest hyalines consist of a mass of blue protoplasm and a clump of carmine-violet stained chromatin. They are 2 to 3 microns in diameter. Soon the “achromatic zone” appears, the “vesicular part” of the nucleus, which may be the largest part of the parasite. The protoplasm now often forms a wide crescentic ring surrounding this, with the chromatin mass between the tips of its horns, but often not quite touched by them. A “milk-white zone” of Gautier often surrounds the chromatin mass, but is not present at all ages, nor in all of the same age. To just what part of this structure the term nucleus shall be applied is disputed. Stephens and Christophers, as well as many previous observers, use the term for the chromatin mass alone, while others include the chromatin mass and milk-white zone, and others also the much larger achromatic zone.

At this point should be emphasized the necessity of seeing distinctly the blue protoplasm and the red chromatin to recognize the malarial hyaline. This is necessary, since so many other structures can look almost like a hyaline, as, for instance, certain degenerations of the red blood-cells, and particularly platelets on the cells (Plate III, 21, 27). These or any other structure on the cell are always surrounded by a colorless zone, probably due to the pressure which they exert on the corpuscle, squeezing the hæmoglobin from their vicinity. It is of interest that in the case of malarial parasites this is not usually the case, but the hæmoglobin is in direct contact with the parasite, good evidence, says Ross, that the hyaline is intracellular and not adherent to the surface of the cell. (Argutinsky, Stephens and Christophers, and others.)

At the end of twenty-four hours there has been an increase in the amount of protoplasm, the achromatic zone is a little larger, but the chromatin is the same in amount, although now in a more irregular nodular mass. The milk-white zone is seen in some. Some forms have two or more such definite nuclei.<sup>101</sup> The nucleus of this parasite is not perfectly specialized but is rudimentary, the nuclear material being scattered in the cell or collected in one or more masses. In the full-grown the chromatin breaks up into a cluster of fine granules occupying a large achromatic zone, both of which just before segmentation seem entirely to disappear. It then reappears in fine granules arranged in strands and masses throughout the protoplasm. These congregate into four or five clusters and then separate into from fifteen to twenty dense round masses. Achromatic zones now appear around each of these masses, the protoplasm collects around them as a centre, and the segments separate. For a description with much more complex details, which tries to bring this division into the same class as all nuclear division, see Argutinsky.<sup>102</sup> The pigment at the beginning of segmentation is pushed to the periphery, and after segmentation is complete, collects in one or two masses near the centre. It will be remembered that in the fresh specimen at the time the pigment went to the centre there was no evidence of the separation into segments, hence segmentation is a process which is really complete before there is any sign of it in the fresh specimen. It is claimed by many that in the stained specimen the gamete generation can be followed from the hyaline form onward. According to Stephens and Christophers the young gamete is characterized by the position of the chromatin, which lies in the centre of the vacuole instead of at the edge as is the rule in the asexual forms. During the cycle the cell is in

<sup>101</sup> For evidence of conjugation, see Ewing, J. H. H. Bull., 1900, and Clinical Pathology of the Blood, 1903.

<sup>102</sup> Arch. f. mikr. Anat. und Entwicklungsges., 1901, Bd. 59, p. 315.

some cases filled with basophile granules (see page 510), "Plehn's karyochromatophilic granules," "Schügnier's granules" (Plate III, 10, 13). Bignami and Bastianelli claim that the division of chromatin into fine granules marks the gamete even in the hyaline stage, but Lazear doubts this, since the pigment always just before segmentation divides into fine granules.

The full-grown macrogamete (Plate III, 14) contains an abundance of protoplasm which stains a deep blue, and a small amount of chromatin in a compact mass which is peripherally placed and surrounded by a thin vacuole-like area, this nucleus occupying about one-tenth the cell. The pigment is uniformly distributed. The remains of the corpuscle often cannot be seen. In the microgametocytes (Plate III, 11) the chromatin is more voluminous, looser, centrally placed in a large achromatic zone. It is in a band arranged as a knot or skein, its thread-like nature always evident. The parasite fills about two-thirds of the cell; its protoplasm is in a ring around the nucleus. It stains a grayish-green or grayish-red color, and not at all the blue of the female form, hence the pigment is easily seen.

QUARTAN (Plate III, 15-18).—The structure of the quartan resembles that of the tertian, but in the hyaline the chromatin mass is less dense, is, in fact, an irregular clump of granules, and in the older forms is in a cluster of fine granules without a distinct achromatic zone, hence often hard and sometimes impossible to see. The parasite is usually a band across the cell.

ÆSTIVO-AUTUMNAL (Plate III, 19-29).—In the hyalines the chromatin is in from one to three masses or filaments. The protoplasm is scantier than in the other forms and remains so throughout the cycle. Characteristic of this form at a later stage is the large oval ring of protoplasm with a thicker layer opposite the chromatin mass. The young gamete forms are characteristic (Maurer). They are accurately spherical, being a ring of the same thickness all the way around. The nucleus forms a portion of the ring, but this does not project as in the schizonts, and the red blood-cell usually presents no coarse stippling (Maurer). Of the crescents, the male form has its chromatin in a loose net-work which occupies the most of the cell, comparatively little blue staining protoplasm, and the pigment scattered throughout its body. This crescent is somewhat kidney-shaped, is shorter and broader than is the female form. The female crescent is quite long and narrow, its chromatin is more compact and more or less centrally placed, there is much more blue staining protoplasm, and the pigment is in a ring around the nucleus or in a clump near the centre.

There are also two types of circular bodies, the microgametocyte, smaller than the red, perfectly spherical, with chromatin in the centre in a large irregular mass like a tangled thread, later in four or

five dense masses near the periphery, which then are extruded as the flagella (or microgametes). Sometimes a thin bluish envelope of protoplasm can be stained enveloping the chromatin thread of the flagellum. The macrogamete is two or three times as large as this, often of triangular shape, with abundant blue protoplasm; the chromatin is in a single mass at the periphery and surrounded by a circle of pigment. In the stained specimens (especially in sections cut in paraffin) can be seen the projection of the chromatin mass and part of the protoplasm apparently from the surface of the cell, hence the belief (Argutinsky, Stephens and Christophers) that it at all stages rests on the cell, not in it (Plate III, 20). Study of the fresh blood, by far the more important, noting especially the way they burst through a fine opening, their amoeboid phenomena, etc., shows conclusively, we think, that they are intracellular.

The following points may be emphasized: In the case of tertian and quartan malaria, organisms and chills are not synonymous. The infection must reach a certain degree (250,000,000 organisms, Ross) before chills begin. Because no parasites are found may not rule out malaria, especially if the patient has been taking quinine. Fevers with long intervals are thus explained, very many parasites being killed off at each chill, hence some delay before enough have reaccumulated to cause a second chill.

In a case of fever to find a few crescents does not mean necessarily that the fever is malaria, since these gamete forms may persist for months after the asexual cycle has stopped. In case hyalines also are found the diagnosis is justifiable, especially if the fever yields promptly to quinine. The asexual cycle is the "febrile cycle." The sexual has no influence over this host, except that it may again start up the asexual cycle, explaining relapses in early spring and especially those occurring after an accident or a surgical operation even two years after any chance of reinfection has passed. Not all the members of the same tertian or quartan group are of exactly the same size or age, and the segmentation continues through at least twelve or fourteen hours. This is fortunate, since, did they segment more in unison, hæmoglobinuria would probably be more common (as analogue, see Texas fever of cattle). The size of the segmenters varies so much that it is supposed that when the majority begin to segment all the others not quite mature as yet are drawn into a "precocious segmentation" (Plate IV, 16, 17). This keeps the groups at an almost equal age, for otherwise these younger forms would disturb the grouping to the degree which occurs in æstivo-autumnal malaria. It also may explain the sudden appearance of a second group in a previously single infection, a few of the forms being so young that they cannot be drawn into this precocious segmentation.

The conditions on a slide under the microscope are more like those in the mosquito's stomach than in the circulating blood, hence many changes (*e.g.*, flagellation, the cadaveric forms) must not be considered as occurring in the human host.

There is a remarkable periodicity in the cycle which we do not understand; among other illustrations, the tendency to flagellate. In some cases used for class demonstration so many flagellated forms will be found that all the students can study the process; in other cases with even more sexual forms not one will flagellate.

The distribution of parasites in the body is remarkable. The æstivo-autumnal lives for the most part in the spleen, liver, and bone marrow; the same to a less degree is true of the tertian. But it is their accumulation in other organs which is important; in the brain and medulla causing thrombi, hence paralyses,

transient aphasias, mental symptoms, even sudden death; and in the mucosa of the gastro-intestinal tract causing even necrosis and sloughing, hence severe vomiting and diarrhoea.

Whether the virulence of the infection depends on the number of parasites or not is hard to answer; it certainly does not on the number in the peripheral blood, although pernicious cases have usually many organisms visible.

**Trypanosomiasis.**—This most interesting disease in man ("sleeping sickness"), which has recently attracted so much attention, is now considered due to an actively motile fish-shaped flagellate, *Trypanosoma gambiense* (Plate II, 21), which can be seen in the blood-plasma, moving with a screw-like motion among the red blood-cells which it scarcely disturbs. It is from two to three times as long as a red blood-corpuscle (18 to 25 microns long, 2 to 2.5 microns wide), with one flagellum anteriorly and an undulating membrane which extends the whole length.

The parasite is to be searched for in the fresh blood specimens with a medium magnification. There are present sometimes many, generally few. They vary much in numbers, often being absent for long periods, even a month or more, and then reappearing in force, even 70 to a cover-slip specimen. The symptoms seem to bear no relation to the number of parasites in the peripheral blood; it may be necessary to centrifugalize to find any. They can most surely be found by puncturing the cervical lymph glands, and are easy to find in the fluid of oedematous areas. Inoculation experiments may be necessary. Stained with a polychrome-methylene blue and eosin mixture they have a rather large red nucleus at about the middle, a centrosome staining intensely in a vacuole-like area very near the blunt posterior end, and a red line of chromatin running down the edge of the undulating membrane and terminating in the red flagellum. The protoplasm of the body takes a blue stain. Various involution forms will, of course, soon be seen in a fresh specimen. The parasite contains no pigment and hence must live on the plasma. It multiplies by longitudinal fission.

For a long time it has been well recognized that this organism was a common and harmless parasite in the blood of fish, amphibians, birds, and rats, and an important cause of disease among horses, cattle, and other domesticated animals in India, Africa especially, and South America. The disease has borne several names. The "tsetse fly disease" of Central Africa caused by *Trypanosoma brucei*, is usually fatal to almost all domestic animals, especially the horse, the mule, and the dog, less so for cattle and still less so for the ass, least for sheep and goats. Man was, however, supposed to be immune. It is communicated by a fly, *Glossina morsitans*. Flies seem to carry it mechanically, and to play no part in its life history.

The "surra," of India, a disease which attacks horses and camels

especially, is caused by a parasite discovered in 1881 by Evans, which differs in no way from *Trypanosoma brucei*. The same may perhaps be true of the parasite of "mal de Caderas" of Central and South America, which attacks especially horses.

The parasite was first discovered in man by Dutton in 1902 in the blood, and in the cerebrospinal fluid of a case of sleeping sickness by Castellani, but it was Bruce who first recognized its pathogenic importance in man. This disease is communicated by *Glossina palpalis*.

Of eighty persons in good health in Uganda, Bruce found the parasite in the blood of twenty-three, but many of these have since died. The parasite may thus be found in men apparently normal for some time, but the present opinion is that it is sooner or later fatal. The symptoms are somewhat like those of malaria. It is a disease which can take a rather acute course, but as a rule is exceedingly chronic, running for years, yet uniformly fatal when the parasite invades the cerebrospinal fluid which seems to be the common or perhaps unfailing result. It is accompanied by an irregular temperature often with intermissions, by multiple erythema, moderate anæmia, marked emaciation, loss of strength, localized œdema of face, trunk, and legs, enlarged spleen, and swelling of the lymph-glands, especially those of the posterior cervical region. Later, the so-called "sleeping sickness" begins, which is due to the presence of the parasite in the cerebrospinal fluid, and found there in practically every case (Bruce).

This fluid should be centrifugalized gently for fully five minutes. It can then be poured off to the last drop into another tube and the sediment examined under a well vaselined cover-glass. If centrifugalized too violently the motility of the parasites will be less, and they may be mutilated by the weight of the sediment. The fluid should be centrifugalized two or even three times, for none may be found in the first sediment. Not many are present.

The parasite of man, *Trypanosoma gambiense*, can in no way be distinguished from that of the tsetse or surra; either morphologically or pathogenically.

There are two other forms of trypanosoma which are easily distinguished from that of man, and which occur quite commonly. The one is *Trypanosoma theileri*, which is pathogenic for cattle alone,—it is a parasite from two to three times as long as the human form,—and the trypanosoma of rats, which is morphologically characteristic, since the posterior end is long drawn out and pointed, the centrosome is not near the end, but at the juncture of the posterior and the middle thirds. It can be easily distinguished from the other trypanosomata, even when they coexist in the blood. It occurs in about 10 to 30 per cent. of rats investigated in some regions, in others in even 90 per cent.



For a recent discussion of the whole subject the reader is referred to the report by Musgrave and Clegg.<sup>103</sup> A recent good brief review is by Koch.<sup>104</sup>

**Piroplasmosis. Infection with the Leishman-Donovan Bodies.**—The Leishman-Donovan bodies (see Fig. 120) are small, oval, round, or oat-shaped bodies, from 2.5 to 3.5 microns in diameter. They have a definite cell outline and contain two chromatin masses, a larger one, the “nucleus,” almost round or oval which stains faintly, and a smaller, bacillus-shaped “centrosome,” which stains deeply, and which is directed either at right angles or nearly so to the axis of the nucleus. These two bodies are both in the long axis of the cell, the larger on the periphery. Many are vacuolated. The outline of the cell cannot always be seen, but these two masses thus arranged are distinctive. They are easily stained by the various polychrome methylene blue-eosin mixtures. They are best studied with the highest powers, the oil lens and V ocular.

They are not found in the circulating blood, except a few intracellulars in two fatal cases, but easily in that from splenic puncture, and in the granulation tissue snipped off from the ulcers with scissors and crushed thin on the slide. At autopsy many are found in the mesenteric lymph-glands, bone-marrow, and liver.

Some lie free but most seem to be intracellular, one or two in a leucocyte (?), from one to twelve in endothelial or splenic cells, some, even hundreds in large masses, in macrophages(?). These masses are variously interpreted. If cells, they are badly degenerated. Ross considers them to be a “matrix” in which the organisms lie, and that none are intracellular, and Manson regards such masses as zoöglia.

Their division may be followed. It begins in the larger chromatin mass and ends in the smaller which may begin to divide after the fragments of the larger are widely separated.

This parasite is supposed to be the cause of some cases of the chronic “malarial” cachexia of the Tropics, dum-dum fever, kala azar, tropical splenomegaly; of the tropical ulcer, Delhi boil, Aleppo button, Scinde sore, oriental sore, etc. It is a filth disease. In the Tropics it promises to prove even more important than the malarial organism. Clinically there are great enlargement of the spleen, emaciation, irregular fever, various abdominal symptoms, and cutaneous hemorrhages and ulcerations.

Donovan reports 72 cases,<sup>105</sup> with a mortality of 30.55 per cent.

The blood features are a moderate anæmia, from 2,000,000 to 4,000,000, and leucopenia, with a relative and absolute increase of the large mononuclears. The average leucocyte count is about

<sup>103</sup> Biological Lab. Department of the Interior, Bureau of Government Laboratories, 1903.

<sup>104</sup> Deutsches med. Wochenschr., 1904, No. 47.

<sup>105</sup> Lancet, September 10, 1904.





FIG. 120.—Leishman-Donovan bodies. From splenic puncture.  $\times 1200$ .



2000. In a case of Neave the large mononuclears were 67 per cent. (total count 3000); pmn. n., 20 per cent.; sm. monos., 11 per cent.; eosin., 1 per cent.; myelocytes, 1 per cent. The patient was an eight-year-old boy. In most cases the formula is more nearly normal.

These parasites were first described in 1900 by Leishman as degenerated trypanosomes, an idea which is now held by some. Whether related or not, it is agreed that these may show flagellated forms, and that degenerated trypanosomes can assume a form similar to this, but those interested refuse to find any relationship.

**Filariasis.**—Of the various forms described in human blood, that most common is *Filaria bancrofti* (*F. nocturna*). These embryos are from 270 to 340 microns long, and from 7 to 11 broad (see Fig. 121). They are enclosed in a sheath which is considerably longer than the parasite. The anterior end of the worm is abruptly rounded, with six-tipped prepuce and sharp fang; the posterior tapers off for two-fifths of its length. It has a granular median axis. At

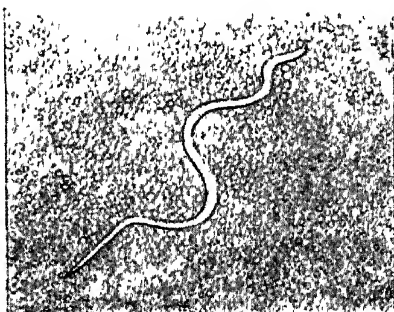


FIG. 121.—*Filaria bancrofti*.  $\times 50$ .

first their movement is progressive, but they seem soon to become attached to the glass at their anterior end, and there they remain, lashing the surrounding corpuscles for days. These embryos appear in the circulation towards evening, their number gradually rising to a maximum at about midnight, then diminishing toward dawn. During the day they are in the internal organs, especially the lungs.

Lothrop and Pratt<sup>106</sup> charted hourly the numbers counted, and found at midnight 2100 per cubic centimetre.

The adults lie in the lymphatics, where they obstruct the lymph flow, causing lymph-scrotum, elephantiasis, occlusion of the thoracic duct, and various other lymph tumors. This obstruction is also attributed to the eggs, which are too wide to pass through capillaries. This is the chief cause of hæmatochyluria. The female is 85 to 150 mm. long, with a distinct neck, a head with simple minute terminal mouth, a plain cylindrical body covered by a striated cuticle, and which tapers to the neck and tail. The tail ends bluntly and has a small depression surrounded by two lips. The anus is a ventral opening on the summit of a trilobed papilla.

The ova are 25 to 38 microns long by 15 microns broad. The females are generally viviparous, but may discharge the eggs. The embryos reach the general circulation through the thoracic duct.

The male is 80 mm. long without neck, and a tendril-like tail rolled up in one or two spirals. The œsophagus is thick walled. The cloaca is ventral, with four pre-anal and four post-anal papillæ and two spicules.

The intermediate hosts are some varieties of *Culex* and *Anopheles* mosquitoes. About an hour after the bite the embryos in the mosquito's stomach cast their sheath. Some die, but others actively bore their way through the intestinal wall to the muscles, where they rest. During the next two or three days the embryo becomes larger and the alimentary tract develops. On the seventh day the worm is 1.5 mm. long and perfectly developed. It now actively travels to the head and takes its position in the labium, whence it enters its new host during the biting by piercing the delicate membrane of the end of the proboscis. It takes an infection by even hundreds of these adult forms to cause a very severe case, and it may be years before any symptoms begin.

The clinical symptoms, in addition to the various lymph tumors, are anæmia, enlarged spleen, and fever. In any case of lymph tumor, elephantiasis, hæmato-chyluria, the blood should be examined. These cases are usually admitted to the surgical side, and an interesting number have been operated on for inguinal hernia, the lymph-scrutum being thus interpreted. Probably there are a good many cases in this country, judging from the number recently found in quite widely distant cities.

It occurs endemic in the Tropics. In the Fiji Islands as much as 25 per cent., and in the Friendly Islands even 32 per cent., of the inhabitants are said to be infected with this disease, also called "craw-craw," or the "sleeping disease."

The blood should be examined late at night. A very thick fresh specimen is made and examined with the low power. These worms cannot be overlooked. Their motion will continue for even a week in a well-sealed specimen.

The *hæmatochyluria* is due to rupture of the varicosed lymph-vessels of the bladder, these forming much of the collateral circulation which compensates for an occluded thoracic duct. The attacks may occur for even eighteen years, each being weeks or months long and separated by intervals of months or years. They come on spontaneously or following exertion, excitement, etc. The onset is with pain and fever. The sequence is, hæmaturia, hæmatochyluria, chyluria. In the urine are found embryos. The urine contains most blood and embryos in the early morning, most chyle after a rich meal (even 3.8 per cent. fat). (For the blood formula, see page 565.)

*FILARIA DIURNA* (the embryos) differs little from *Filaria nocturna* (bancrofti), except that it remains in the circulation only during the day, and the adult form is not yet known (*Filaria loa* of the subcutaneous areolar tissue?). The granular axis fails.

*FILARIA PERSTANS*.—These embryos are about 200 microns long and 4 to 5 in width, without a sheath, and with very active, progressive, as well as lashing motion. They remain in the circulation day and night. The body tapers for its posterior two thirds; it has a slightly bulbous tail.

The adult is situated in the retroperitoneal tissue.

Other forms described in man are *Filaria ozzardi* (embryos small, 170 to 200 microns long, without sheath and with sharp tail, no periodicity, its adult in the sub-

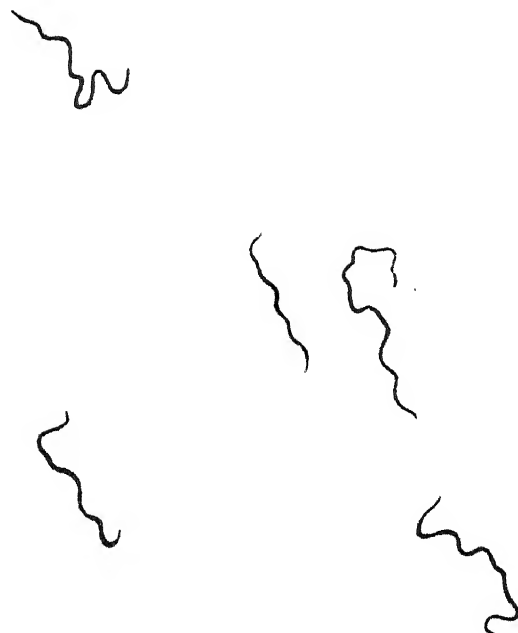


Fig. 122. - The sporocyst of relapsing fever. 1000x.



peritoneal tissue); *Filaria demarquai* (embryos 200 microns long, sheathless, and sharp-tailed, with cephalic armature, no periodicity, adult doubtful); *Filaria megalhæsi*, *Filaria gigas*, and *Filaria loa*.

**Relapsing Fever.**—The organism which causes the relapsing or famine fever of Europe is *Spirochæte* (or *Spirillum*) *obermeieri*, an organism curled like a corkscrew and from 12 to 45 microns in length and from 0.3 to 0.5 micron in breadth. Its curves, from four to sixteen in number, are sharp and regular. Its ends are pointed. It is flagellated, but there is a dispute as to the number of flagella. This organism is present in large numbers in the circulating blood of patients from the onset of the fever until the crisis, when it suddenly disappears. At the beginning of the attack the parasite moves with rapid corkscrew motion among the blood corpuscles, which are not much disturbed by the motion; later, as if the individual parasites were losing their vitality, there is simply an undulatory movement of the whole spirochæte, while still later they make merely a slight swaying motion. After the crisis, it is said, the parasites collect in the spleen. Little is known of the life history of this organism outside the human body. Relapsing fever is certainly a filth disease, and the bed-bug is accused of being the agent of its natural transmission, but this is not yet proved.

Three similar organisms have been discovered.\* These are *Spirillum duttoni*, the cause of the African disease; *Spirillum carteri*, cause of the Asian disease; and *Spirillum novyi*, cause of the American disease.

*Spirillum duttoni* varies in length from fifteen to forty-five microns, and in width from 0.2 to 0.4 micron. It has from two to six curves. Whether it has flagella and undulating membrane is disputed. Not nearly as many of these *Spirilla* are present in the blood of patients during attacks as are present of the other organisms in other spirillar fevers. Transmission to man seems to be by ticks.

*Spirillum novyi* is an organism whose length is from 7 to 9 (or multiples of this) microns. It is shorter and finer than the other spirilla and has two or three sharp regular curves.

*Spirilla carteri* is a parasite from 12 to 16 microns long by 0.3 to 0.5 micron wide. The infection which this organism causes and which lice is supposed to spread is severe.

**Trichinella Spiralis** has been found in the circulating blood very few times. It was found there first by Herrick and Janeway,† using Staubli's method. (The blood, from a few drops to 10 cc. in amount, is laked with from 10 to 15 parts of 3 per cent. acetic acid. This laked blood is centrifugalized, and the sediment examined.)

\* Mackie, N. Y. Med. Jour., Aug. 22, 1908.

† Arch. of Int. Med., April, 1909.

## OPSONINS.\*

Phagocytosis has long been looked upon as one of the strong defences of the body against infection. It has been observed that as a rule bacteria are ingested by the phagocytes more readily in the presence of serum than in its absence. This action of the serum in facilitating phagocytosis is referred to the presence in the serum of a hypothetical "body" to which the term "opsonin" has been applied and which is supposed to act upon the bacteria, producing some change in them which facilitates their ingestion by the phagocytes.

That the action of the serum is directed toward the bacteria rather than the phagocytes is indicated by the following results: Bacteria which have remained in contact with serum at  $37\frac{1}{2}^{\circ}$  C. for a short time and then repeatedly washed in normal salt solution to remove the serum, are readily ingested by washed leucocytes, while bacteria which have not been exposed to the action of serum are ingested to a much less extent by washed leucocytes. This is not universally true of all varieties of bacteria. Some, such as *Bacillus pyocyaneus*, may be readily ingested by the leucocytes without previously having been acted on by serum, and a greater or less amount of phagocytosis of practically any variety of bacterium occurs independently of the action of serum, so-called spontaneous phagocytosis. Spontaneous phagocytosis is said to be inhibited by a 1.2 per cent. concentration of sodium chloride. Not only do different varieties of bacteria differ in their resistance to phagocytosis, but different strains of the same variety may show a marked variation in their resistance to phagocytosis even under the influence of the same serum. In general the more virulent the strain the more resistant it is to phagocytosis.

Opsonins are placed in the category of immune bodies along with agglutinins, precipitins, bacteriolysins, etc., and like them are regarded as specific bodies; that is, just as the agglutinins for different varieties of bacteria are specific so there are specific opsonins. Like the agglutinins also, opsonins occur normally in the serum and as a result of infection or immunization, the former being designated as normal opsonins, the latter as immune opsonins. Normal opsonins are said to be thermo-labile, being destroyed by an exposure to  $57^{\circ}$  C. per half hour, while immune opsonins resist this exposure and are therefore thermo-stabile.

If the foregoing observations are correct and if opsonins are really specific immune bodies, playing a very important part in the defensive mechanism of the body, any method which would enable their accurate estimation might be of great service in diagnosis and

\*For the following two sections I am indebted to Dr. Wm. L. Moss.



prognosis and any means of regulating their presence in the body might be of great therapeutic importance. Unfortunately, notwithstanding a vast amount of knowledge concerning all the "immune bodies," investigators do not yet know upon what immunity depends.

For diagnostic purposes the estimation of any of the other immune bodies—agglutinins, precipitins, complement-fixing amboceptors, etc.—is probably more valuable than the opsonic index. Prognostically little value can be attached to any of them, and by therapeutic measures any of these bodies can be more influenced than can opsonins. Nevertheless, efforts are still being made to use the opsonic index for both diagnostic and prognostic purposes and as a control for the administration of vaccine therapy.

The power of the serum in favoring phagocytosis is spoken of as its opsonic power. The opsonic index of a given individual is the ratio of the opsonic power of his serum to the opsonic power of the serum of a normal individual.

To determine the opsonic power of a serum, equal quantities of serum, leucocytic suspension and bacterial suspension are mixed and incubated at  $37\frac{1}{2}^{\circ}$  C. Smears are then made from the mixture, and after appropriate staining the average number of bacteria ingested per leucocyte is determined. This number represents the opsonic power of the serum and is sometimes spoken of as the phagocytic index.

Thus the ratio which the phagocytic index of a given serum bears to the phagocytic index of a normal serum is the opsonic index.

For example: Suppose the average number of bacteria taken up by the leucocytes in a preparation in which patient's serum has been used is 3, and the average number taken up by the leucocytes in a preparation in which normal serum has been used is 6; the ratio is 3:6. The opsonic index expressed by this ratio is, therefore, 0.5. Had the average number in the preparation in which patient's serum was used been 9, the ratio would have been 9:6, and consequently the opsonic index 1.5.

The technique described below is essentially that used by Wright, but as the sources of error are so great and so numerous, even with the best technique, certain refinements have been added. For the sake of definiteness the technique will be described in great detail, although it will readily be understood that certain modifications might be made. The technique may, perhaps, best be described by taking a specific case, and as the *Staphylococcus aureus* is one of the easiest organisms with which to work, we will consider that one. The following preparations are necessary:

1. A suspension of leucocytes (obtained from any source) free from serum.
  2. A suspension of *Staphylococcus aureus*.
  3. A specimen of normal serum.
  4. A specimen of the patient's serum.
1. Preparation of leucocytic suspension: A clean, sterile centrifuge tube is

filled three-fourths full of a sterile solution containing 1.5 per cent. sodium citrate and .85 per cent. sodium chloride. The dorsal surface of the distal phalanx of the index or middle finger of the left hand is now cleansed with alcohol and wiped dry, the hand is swung briskly to and fro several times to congest it, and before the excess of blood has time to escape from the hand, a bandage, or piece of small rubber tubing, is wound tightly around the prepared finger, beginning at the base and extending down to or past the middle joint. (See Fig. 122a.) With a sharp blood sticker a prick is made in the cleansed area and ten drops of blood are allowed to flow into the centrifuge tube containing the sodium citrate solution. This is immediately mixed by drawing it up several times into a sterile 10 cc. pipette. The tube is now centrifugalized for about three minutes at a speed of 1500 to 2000 revolutions per minute. The red corpuscles, being heavier, will occupy the bottom of the tube and will be covered with a very thin layer of leucocytes. The supernatant fluid will be clear or very slightly turbid from the still suspended platelets, or sometimes, perhaps, from the fat, etc., contained in the serum.

By longer centrifugalization the platelets can also be thrown down, but this is not desirable. Better and cleaner preparations are obtained if the platelets are not included. With a little experience one learns to judge by the degree of turbidity of the supernatant fluid when to stop the centrifugalization so as to get a maximum number of leucocytes with a minimum number of platelets.

The supernatant fluid is now removed by means of a sterile pipette. For this purpose a 10 cc. pipette of the Mohr pattern fitted with a rubber bulb of 10 or 15 cc. capacity is very convenient.

The centrifuge tube is now filled to within one or two centimetres of the top with sterile .85 per cent. salt solution and the corpuscles thoroughly mixed again by means of the pipette. The tube is replaced in the centrifuge, the corpuscles again thrown down, and the supernatant fluid removed. This washing with salt solution is repeated still again.

After the third centrifugalization (once in sodium citrate solution and twice in sodium chloride solution) the supernatant fluid is pipetted off down to within one centimetre of the surface of the corpuscles.

The point of a capillary pipette is now introduced down to the surface of the corpuscles through the one centimetre of supernatant fluid and by means of a rubber teat the layer of leucocytes, together with a few red cells, may be drawn off and transferred to another tube. A small test tube, measuring  $\frac{3}{4} \times 7$  cm., is convenient for this purpose.

The leucocytes which have been transferred in this way, together with the few red cells and small amount of sodium chloride solution necessarily brought over with them, are thoroughly mixed just before using by drawing into and expelling from the capillary pipette used in their transference. Care must be exercised to produce no air bubbles in this process.

2. The preparation of the suspension of bacteria: A twenty-four-hour slant agar culture of *Staphylococcus* is used for this purpose.

Into a watch glass or small test tube are poured about three cubic centimetres of .85 per cent. sodium chloride solution. Some of the growth is removed from the culture tube with the platinum loop and is thoroughly rubbed up on the side of the watch glass (or small test tube) just above the level of the salt solution, the glass having previously been wet with the fluid by tilting. In this way the bacteria are gradually washed down into the salt solution. This process is repeated until the emulsion is slightly turbid. It should contain no clumps.

To judge the proper degree of turbidity is a matter of experience. It is desirable to have a suspension which, when incubated with leucocytes and serum, will yield an average of about six bacteria per polymorpholeucocyte. It will probably be necessary to test this by trial preparations. If the leucocytes are found to contain too many bacteria, the suspension should be diluted; if too few, more bacteria should be added to the suspension.

The securing of a good and uniform suspension is further facilitated in the fol-

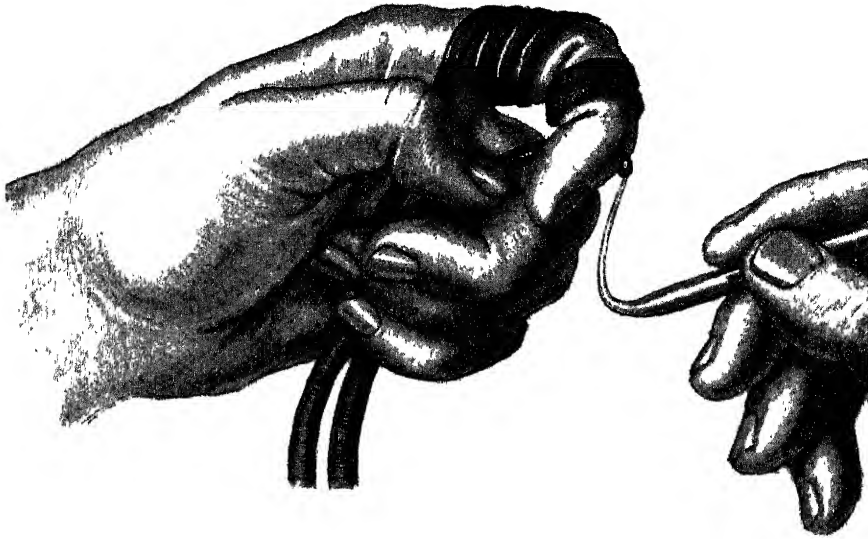


FIG. 122a.—Method of obtaining blood

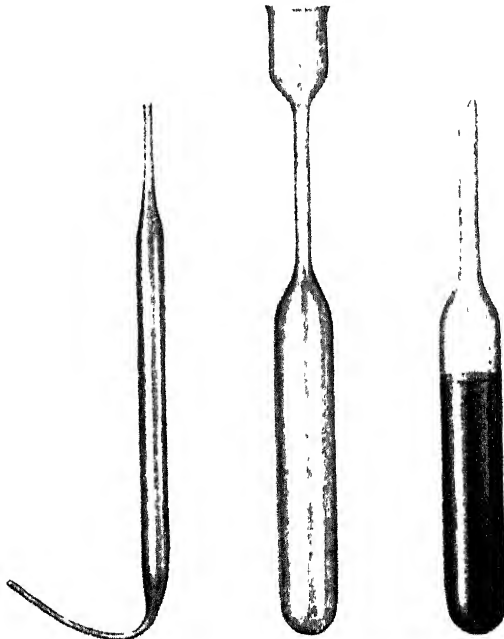


FIG. 122b.—Tubes used in serum work. The tube to the left is used to collect blood. The others for bacterium emulsions. (Reduced just one-half.)



FIG. 122c.—Smear of the spinal fluid of a case of epidemic cerebrospinal meningitis.



FIG. 122d. Smear of the spinal fluid of a case of meningitis due to *Diplococcus lanceolatus*.

lowing way: A capillary pipette fitted with a rubber teat and with the capillary end cut squarely off, is pressed evenly against the bottom of the watch glass and the suspension drawn in and forced out of the tube a number of times. In passing through the narrow chink left between the bottom of the pipette and the watch glass, the small clumps are usually broken up.

To produce a satisfactory suspension one should use a rather moist growth of bacteria. Such a growth may be facilitated by using culture tubes containing a fair amount of water of condensation and a medium which is not too solid. Should difficulty be experienced, however, in getting rid of clumps, one may resort to the following additional procedure: A suspension is made as above except, perhaps, slightly thicker. This is now centrifugalized for two or three minutes at 1500 to 2000 revolutions per minute and the supernatant fluid, now free from clumps, pipetted off and used.

3. The method of obtaining the serum: The serum is most conveniently obtained in glass tubes of the form shown in Fig. 122b. With a little practice these are easily made from ordinary glass tubing of about five millimetres internal diameter. They should be about ten centimetres in length.

The blood is gotten from the finger as described for obtaining the leucocytes. (Fig. 122a.) The curved end of the tube is touched to the drop of blood, the body of the tube being held at a lower level, but slanting somewhat, so that the blood will not run into the straight end. Having filled the tube half or two-thirds full, the straight end is warmed gently to expel some of the air, and then the point is sealed in the flame. As the tube cools the blood will be drawn from the curved end toward the straight end of the tube. Great care must be taken that in warming the tube the blood is not heated and that it does not run up into the straight end of the tube while it is still hot. When the clot has begun to form, the tube is placed in the centrifuge with straight end down and a few minutes' rotation serves to separate the serum.

When ready to use, the tube is marked across with a file about one centimetre above the level of the serum and broken off.

Having now prepared a suspension of leucocytes, a suspension of bacteria, the specimens of the normal and of the patient's serum, the mixtures are made for phagocytosis.

For this purpose one requires capillary pipettes, which are best made from glass tubing about five millimetres in diameter. The tubing is cut into about ten centimetre lengths, heated in the middle and drawn out into capillaries of about one millimetre diameter. These are now broken in two and the capillary ends cut off squarely at such a point as will leave the tube about twenty centimetres long over all. With a glass-marking pencil a fine mark is made on the capillary stem about two and a half centimetres from the small end. By means of this pipette the leucocytes, bacteria, and serum are measured and mixed as follows: The end of the pipette is placed in the leucocytic suspension and by means of a rubber teat the capillary is filled up to the pencil mark. A small bubble of air is now admitted, and the capillary again filled up to the mark with bacterial suspension, the leucocytes, meantime, having been drawn farther up the capillary stem. Another bubble of air is now admitted and the capillary filled up to the mark with normal serum. We now have in the pipette equal volumes of leucocytic suspension, bacterial suspension, and serum, separated by two bubbles of air. The whole is now expelled on a perfectly clean slide and thoroughly and rapidly mixed by repeatedly drawing up into the capillary tube, care being exercised to avoid air bubbles. After thoroughly mixing, the whole volume is drawn up into the capillary so that the lower end of the fluid is about three or four centimetres from the capillary end of the tube, which is now sealed off in the flame, the tube numbered or lettered to designate the serum used, and placed in the incubator at  $37\frac{1}{2}^{\circ}$  C.

A similar preparation is made in which patient's serum is used instead of the normal. Both preparations are kept in the incubator exactly the same length of time, fifteen minutes being the period adapted for most organisms.

Preparation of the blood smears: After removing the pipette from the incu-

bator it is convenient to use a rubber teat with a hole in the convex end for expelling and again mixing the fluid. The hole is readily made with a hot platinum needle. Such a teat is fitted over the open end of the pipette and the capillary end is then cut off. The fluid can now be pressed out on a clean slide by placing a finger over the hole in the teat. After mixing the fluid well by drawing it into the pipette several times, a small drop, about one and one-half to two millimetres in diameter, is placed about two centimetres from the end of a perfectly clean slide. The spread is made with the end of another slide. The corner of the spreading slide is broken off so that the smear will not extend quite to the edges of the first slide. The angle at which the spreading slide is held to the other slide, the pressure and the rapidity with which the spread is made, are all factors in the result. Practice alone will enable one to become expert in this.

A very satisfactory stain for most bacteria is Hastings's stain. With this stain the nuclei of the cells are blue, and the protoplasm a light pink. As the bacteria take the stain with more avidity than do the nuclei of the cells, they can readily be counted even when overlying the nuclei, provided care has been exercised not to stain the preparations too deeply.

It is always desirable to make a duplicate preparation from each pipette. This duplicate is numbered and laid aside, usually without staining, and is available should the first slide not prove satisfactory.

If the preparation has been successful, the leucocytes should be found abundantly toward the latter end of the smear. With the low power of the microscope one finds an area suitable for counting. The leucocytes should be well flattened out, the margins easily definable, and the cells not massed together in confusion. In a good preparation one should be able to count several hundred leucocytes, if desired, without spending too much time searching for them. In enumerating the bacteria one is sometimes at a loss to know whether to include bacteria which apparently lie just on the periphery of the cell. It probably does not make much difference whether they are included or not so long as one constantly follows the same rule, but it seems better, perhaps, to include only those bacteria which lie wholly within the periphery of the cell.

*The Count.*—Most workers arbitrarily count only the bacteria included within the polymorphonuclear leucocytes. The number of leucocytes counted varies greatly in different laboratories. The larger the number counted, the more nearly will the result represent the true average. Not less than one hundred cells should be counted in each preparation.

*The Opsonic Index.*—Having obtained the average number of bacteria per leucocyte for the preparation in which patient's serum was used, and the average number for the preparation in which normal serum was used, the opsonic index is obtained by dividing the patient's average by the normal's average.

*The Technique for Other Bacteria.*—The technique has been described for *Staphylococcus* because it is one of the simplest organisms with which to work. The technique is practically identical for the *Gonococcus*, *Bacillus typhosus*, *Bacillus coli*, etc., except that for the latter two, a somewhat shorter incubation period, ten minutes, may be necessary, as many of the bacteria ingested may be digested by the leucocytes in fifteen minutes.

It is necessary to say a word about the estimation of the tuberculo-opsonic index, the preparation of the bacillary suspension and the staining being different from that above described.

*The Bacterial Suspension.*—On account of the danger of working

with living tubercle bacilli, it has been customary to use the dead germs in opsonic determinations.

For preparing the suspensions one may obtain the dead, dried, and ground bodies of tubercle bacilli from the manufacturers of tuberculin, or one may sterilize a living culture by heating. Both of these procedures are open to objections. In the former one encounters many fragmented bacilli, which lead to error in estimating the index, and by the latter procedure, sterilization by heat, the bacilli are so clumped as to render the subsequent production of a uniform suspension very difficult.

Jeans and Sellards\* have suggested a satisfactory method of preparing a suspension of tubercle bacilli which is as follows: Into a thick-walled test-tube some of the growth from a living culture is placed. This is thoroughly ground against the sides of the tube by means of a glass rod, until a perfectly smooth paste is obtained. Salt solution (0.85 per cent.) is now added, drop at a time, while the grinding is continued until the desired degree of turbidity is obtained. Sterilization is accomplished by exposing the suspension to direct sunlight for 10 hours. A drop of the fluid is now examined under the microscope to see that there are no clumps of bacilli present. Should a few clumps be found, they may be gotten rid of by centrifugalizing or by allowing the suspension to sediment for a few days in a narrow cylinder and then removing the still somewhat turbid supernatant fluid, which should be free from clumps.

It is usually stated to be difficult to obtain a uniform suspension of living tubercle bacilli. By selection, however, strains of tubercle bacilli can be found which are easily suspended, or one may follow the methods described for producing homogeneous cultures of tubercle bacilli. Tubercle bacilli should not be sterilized by heat after being suspended, as the heat causes them to clump again.

*Staining Tubercle Preparations.*—The blood smears are made in the same way as described above for *Staphylococcus*. They are fixed either by heat, methyl alcohol, or by immersing for one or two minutes in a saturated solution of bichloride of mercury. They may then be stained in hot carbolfuchsin for five minutes or placed in a staining dish, covered with carbolfuchsin and allowed to stand over night in the incubator. Decolorization is accomplished with 95 per cent. alcohol, and counterstaining with an aqueous solution of methylene blue. The red cells may be gotten rid of before staining by immersing the slide in dilute acetic acid, but if the blood smear has been properly made, this will not be necessary.

*Other Methods of Estimating the Opsonic Index.*—The above technique is, perhaps, the one most extensively used in the estimation of the opsonic index, but various other methods have been proposed and used.

It is not necessary to work with such minute quantities of material as described for the capillary method, although it is convenient. Neither is it necessary to combine the leucocytic suspension, bacterial suspension, and serum in equal quantities. Instead of using capillary pipettes one may measure the suspensions and serum in standard one-cubic centimetre pipettes graduated into one-tenths, and incubate the mixture in small test tubes. On account of the usual difficulty of obtaining large quantities, especially of leucocytes, it is not convenient to employ more than

\* Johns Hopkins Hospital Bulletin, 1907, xviii, Nos. 195-196, p. 233.

0.2 to 0.3 cubic centimetre of each in the mixtures. In preparations made in this way the leucocytes usually settle to the bottom of the tube during incubation and may be removed in a mass with the platinum loop when one makes the smears. The leucocytes thus removed are spread on the slide with the loop and may then be stained and counted as described above.

*Method of Dilution.*—A series of progressive dilutions of the serum may be made and preparations put up from each of the various dilutions, and that beyond which phagocytosis practically ceases, noted. By a comparison of the degree of dilution beyond which phagocytosis ceases for normal and for patient's serum, an opsonic index can be calculated. *E.g.*, if phagocytosis ceases beyond a dilution of 1-100 for normal serum and 1-50 for patient's serum, the opsonic index of the patient would be 0.5.

*Controlling the Normal.*—Instead of taking the serum of a single individual to represent the normal, it is much better to use several normal sera and take the average of all the normals as the normal figure.

The practice of "pooling" the normals; *i.e.*, mixing all the normal sera and then putting up a preparation from such a mixture, is to be condemned. Such a method of control has no value. A separate preparation should be made from each normal serum and the count on each of these should come out within certain narrow limits of each other. The highest count on any one normal serum should not exceed the lowest count on any other normal by more than 15 per cent., and 10 per cent. would be a safer limit.

The partisans of opsonins hold to their specificity; *i.e.*, the index shows a disturbed relation only for that organism which is responsible for the infection. *E.g.*, a patient having a pure *Staphylococcus* infection will have a normal index toward all organisms except *Staphylococcus*. If a patient has an infection of unknown etiology, and his index is tested toward a number of organisms and is found normal to all except one, the diagnosis can be made of infection with that organism for which the abnormal index exists.

*Normal and Immune Opsonins.*—As stated above there are two kinds of opsonins, one thermo-labile, the other thermo-stabile. The former is destroyed by heating to 57° C. for one-half hour. Certain investigators hold that the normal opsonins are thermo-labile and non-specific, while the immune opsonins are thermo-stabile and specific. If this view is correct it would be advisable to inactivate the sera before attempting to use the opsonic index as a diagnostic aid.

*The Value of the Opsonic Index.*—Wright claims that in normal individuals the opsonic index is, within limits, constant. He allows a variation between .8 and 1.2 to cover the normal variation in the opsonic index plus errors in technique. An index below .8 or above 1.2 is taken to indicate infection, and it may happen that an individual with an infection will give an index within the above limits, for he may have been caught just as he was crossing the normal line in passing



from a low to a high, or a high to a low index. Wright lays great stress on an index varying from time to time, as indicating infection.

Considerable work has been done to test the practical value of the opsonic index. The experience at the Johns Hopkins Hospital may be found in the *Johns Hopkins Hospital Bulletin*, June-July, 1907, and attention is called to "An Inquiry into the Value of the Opsonic Index," by Fitzgerald, Strangways and Whiteman, in the *Bulletin of the Committee for the Study of Special Diseases*, Vol. 1, No. 8, August, 1907. At present the status of the opsonic theory seems unsettled. The technique usually employed seems too inaccurate to be relied upon as a diagnostic aid, or as an indication for the treatment by means of vaccines.

Whatever method is followed, care should be taken to use a practically aseptic technique, and that the leucocytes be obtained as short a time as possible before using.

It is usually stated that the serum may be used any time within twenty-four hours, but it seems desirable to make the test within a few hours after the specimen is taken.

It is of the utmost importance that anyone who takes up opsonic work should carefully test the accuracy of the technique for himself.

#### THE WASSERMANN REACTION.

In 1906 Wassermann, Neisser, and Bruck \* published a new test for the diagnosis of syphilis. This test was based upon a phenomenon described by Bordet and Gengou,† as the "fixation of complement." At first it was thought that the new test was a direct application to the diagnosis of syphilis of the principles discovered by Bordet and Gengou; subsequent investigations, however, have indicated that the Wassermann reaction, as it is called, is not specific in a biological sense as is the Bordet-Gengou phenomenon. While this alters the original conception of the reaction, it detracts nothing from its well established value in the diagnosis of syphilis.

A detailed description of the phenomena of immunity cannot be undertaken here, but as some knowledge of the principles upon which the reaction is based and the nomenclature employed in describing them is necessary for a clear understanding of the test, a brief account of these will be given before the technique of the test is described.

As the result of most infections, natural or artificial, or the artificial introduction of any foreign protein substance into the animal

\* Wassermann, A., Neisser, and Bruck: Eine serodiagnostische Reaktion bei Syphilis. Deut. Med. Woch., 1906, xxxii, 745.

† Bordet and Gengou: Sur l'existence de substances sensibilisatrices dans la plupart des sérums antimicrobiens. Ann. de l'Inst. Pasteur, 1901, xv, 289.

jody, the serum of the affected animal acquires new properties which are probably protective or conservative in character.

These properties are ascribed to the presence in the serum of hypothetical "bodies" which are designated as antibodies or immune bodies. Any substance which, on infecting an animal or on being injected into it, stimulates the formation of antibodies is termed antigen. The process of treating animals with antigen in such a way as to bring about the formation in the animal of antibodies, is known as immunization.

There always exists an affinity between antigen and its corresponding antibody, so that if the two are brought together under the proper conditions, they unite in such a way as to alter some of the properties of both.

The relations existing between antigen and antibodies are specific; that is to say, antibodies which result from the injection of a given antigen have an affinity for that particular antigen and no, or only comparatively slight, affinity for any other antigen.

Antibodies, as is well known, are of various orders; for example, antitoxins, agglutinins, precipitins, lysins, etc.

It is logically certain that each antibody is the result of stimulation to the cells of the tissues by a separate antigen, but just as the antibodies have never been isolated in pure state, so the antigens cannot be isolated in pure state and oftentimes cannot be separated from each other. Thus typhoid bacilli probably contain several antigens corresponding to the several antibodies; the agglutinins, precipitins, bacteriolysins, etc., which result from their injection.

Certain of the antibodies are able, directly and unaided, to effect observable changes in their antigens, while others produce no directly observable effect on their antigens, but so change them that a third substance, complement, normally present in serum can bring about profound and observable changes in the antigens.

An example of the first mentioned kind of antibody is agglutinin, which by union with its antigen brings about the agglutination of the cell or bacterium containing it, a directly observable phenomenon. The second kind of antibody is known as amboceptor. The union of an amboceptor with its antigen produces no visible change in the cell containing it; for example, a bacterium or red blood cell, after the union with its appropriate amboceptor, appears just as it did before, but that it has been modified is evidenced by the fact that the addition of complement, a normal constituent of serum, now brings about the solution of the cells.

Within limits complement has no affinity for either antigen or amboceptor alone, but does unite with the complex which results from the union of antigen and amboceptor.

There has been in the past much discussion concerning the unity or plurality of complement. It is now generally conceded that there is more than one kind of complement, perhaps a considerable variety of complements, but the relations existing between complement and an antigen-amboceptor complex are nothing like so specific as those existing between antigen and amboceptor or other antibody. For example, the typhoid amboceptor will unite only with the typhoid bacillus, while the colon amboceptor will unite only with the colon bacillus (exceptions unessential in this connection), but the same complement will unite equally well with either of the above complexes, resulting in the solution of the typhoid bacillus in one case and the colon bacillus in the other.

This specificity of relations existing between antigen and antibody and at least partial non-specificity of those of complement, must be borne in mind, as the Bordet-Gengou phenomenon depends on them.

It has been previously stated that certain of the antibodies, as agglutinin, can directly and unaided produce visible changes in the cells containing the corresponding antigens and that others, as hemolytic or bacteriolytic amboceptors, do not produce visible changes in the cells containing their antigens, until complement is added. There are antibodies which act directly and unaided upon their antigens, for the completion of whose action complement is not necessary and yet which produce no visible change in the antigen. The reaction of toxin and antitoxin is of this character. The change which antitoxin brings about in toxin is not visible. It may be that the toxin molecule combines with the antitoxin molecule to form a new substance whose properties are neither those of toxin nor antitoxin, but none of these bodies have been isolated in pure form, so that no chemical investigation can reveal the change which antitoxin has produced in toxin, and one must resort to animal inoculation in order to prove that a change has taken place.

There probably are immunity reactions of the antigen-amboceptor kind which require complement for their completion, or at least which use up complement if it is present, and yet which produce no visible change to indicate that a reaction has taken place. In such cases some indirect method must be resorted to in order to demonstrate that a reaction has taken place.

Bordet and Gengou proved that complement was used up or "fixed," as they called it when it was added, under proper quantitative relations, to a mixture of bacteria and the corresponding immune serum. In order to prove that complement had disappeared from such a mixture, they added red blood-cells and the appropriate hemolytic amboceptor. If complement had remained in the mixture the red blood-cells would have been dissolved, a phenomenon directly

observable. The fact that they were not dissolved was taken as evidence that the complement had disappeared from the mixture and this phenomenon they described as the "fixation of complement."

Remembering the specific relations of antigen and antibody, it will readily be seen that the phenomenon of fixation of complement is applicable to the identification of an antigen if one has in hand the corresponding amboceptor, or to the identification of an amboceptor if one has in hand the corresponding antigen. It is only necessary to show that complement disappears from a mixture containing the two to prove that the antigen and amboceptor correspond and this is readily done by adding red blood cells and their hemolytic amboceptors.

If one is dealing with antibodies like hemolytic amboceptors or even bacteriolytic amboceptors where the change produced on the cells containing the antigen is directly observable on the addition of complement to the mixture, one does not have to show that complement has been used up in order to prove that a reaction has taken place, since the solution of the cells proves this and this can be directly observed, but if the reaction is between bodies which do not give rise to a visible phenomenon, some such reaction as the test for the fixation of complement is necessary to prove that union between antigen and antibody has taken place.

Wassermann attempted to apply this principle to the diagnosis of syphilis. He reasoned by analogy from other infectious diseases in which antibodies had been demonstrated, that they might occur in the blood of persons infected with syphilis. It seemed not unlikely that if the syphilitic antigen could be obtained the presence of the antibodies could be demonstrated by the ability of the antigen-amboceptor combination to fix complement.

Wassermann sought to obtain the antigen in watery extracts of the spleen and liver of syphilitic fetuses. If such extracts, when added to the serum of a syphilitic patient, were able to fix complement it would be strong evidence that the serum contained specific syphilitic antibodies and the demonstration of such antibodies in the serum of a patient would presumably be of as much value in the diagnosis of syphilis as is the demonstration of typho-agglutinins in the diagnosis of typhoid infection. As an indicator for the presence or absence of complement, in other words to determine if complement had been fixed or not, he used red blood cells and their corresponding hemolytic amboceptor. His results, as is now well known, yielded a most valuable means of diagnosis for syphilis.

Subsequent investigations have shown that the reaction is probably not specific in a biological sense, but this detracts nothing from its practical value. It has been shown that extracts can be prepared from certain organs of non-syphilitic persons and normal animals

which are capable, on the addition of syphilitic serum, of fixing complement, and that even certain lipoids and salts are capable of fixing complement when added to a syphilitic serum.

The method originally devised for performing the test has undergone many modifications at the hands of numerous subsequent investigators, mostly with a view to simplifying it, but in part also to overcome certain defects which have been urged against it. Space does not permit that all of these be discussed here and it is to be feared, in some of these at least, that reliability has been sacrificed to brevity.

In the following pages but two methods of performing the test will be described. The first is essentially that of Wassermann,\* the second is Noguchi's modification.

For carrying out the test the following five preparations are necessary :

1. Extract containing antigen.
  2. Serum to be tested.
  3. Complement.
  4. Hemolytic serum.
  5. A suspension of red blood corpuscles.
- A practically aseptic technique should be used.

1. **Preparation of Antigen.**—The liver of a syphilitic foetus is removed aseptically and minced very fine with sterile scissors or passed through a meat grinder. To this is added absolute alcohol in the proportion of 10 cc. of alcohol to each gram of liver and the mixture shaken on a machine 2 or 3 hours a day for three days, after which it is allowed to sediment until clear and the perfectly clear yellow supernatant fluid is decanted. This may be kept in the refrigerator in rubber-stoppered flasks without undue deterioration for two years or longer. Before using, the antigen must be titrated by a process which will be described later.

In order to ascertain if the liver used is from a syphilitic foetus it may be examined in the fresh state by means of the dark-field illumination for *Triponema pallidum*, or these may be looked for in a preparation stained by the Levaditi method, or a Wassermann reaction may be performed on serum obtained from the foetus if one has a known syphilitic antigen with which to do it.

2. **Method of Obtaining Serum.**—The patient's arm is cleaned up as for a blood culture, and by means of a syringe, 5 cc. of blood are obtained from one of the veins at the bend of the arm. This is placed in a sterile centrifuge tube and allowed to clot. The clot is

\* The Wassermann method is here described as it is at present used in the Bacteriological Laboratory of the Johns Hopkins Hospital.

separated from the walls of the tube by means of a sterile platinum needle and the separation of the serum is facilitated by centrifugalization. This is best done immediately after the clot has formed, before it becomes too solid. The serum is removed from the tube by means of a sterile pipette, care being exercised not to get any blood-corpuscles with it. It is placed in a small test-tube and inactivated by heating it in a water bath of 57° for thirty minutes. This inactivation consists in destroying the complement which the serum contains.

**3. Method of Obtaining Complement.**—Complement is obtained from an extraneous source in order that the amount introduced in all of the tests may be the same.

A normal guinea-pig is anaesthetized, the carotid artery isolated, opened and the blood collected in a sterile centrifuge tube. The serum is separated as described above for patient's serum. In this case, however, it is not heated, as that would destroy the complement.

A more economical method consists in aspirating a few cubic centimetres (up to 5 or 6) of blood directly from the guinea-pig's heart by means of an aspirating syringe. With a little practice this is easily done without sacrificing the pig, which can be used repeatedly for this purpose, if an interval of a week or two is allowed to elapse between bleedings.

The complement may be preserved as long as a month by keeping the serum in an ice chamber at 0° to -5° C. At this temperature the serum remains frozen. If an ice chamber is not at hand, fresh normal serum must be obtained each time the test is made, as complement is an extremely labile body, and disappears when the serum stands for a day or more exposed to light and room temperature.

**4. The Hemolytic Serum.**—This is obtained from rabbits immunized against sheep-corpuscles. A strongly hemolytic serum may usually be obtained by giving rabbits four or five intraperitoneal injections of washed sheep-corpuscles with intervals of four or five days between injections, beginning with a dose of 2 or 3 cc. and gradually increasing to a final dose of 10 or 12 cc. Eight or ten days after the last injection a few cubic centimetres of blood are obtained from an ear vein of the rabbit and the serum tested for its hemolytic strength against sheep-corpuscles. If it is found to cause hemolysis in high dilutions (at least in dilution of 1-500 and better if in higher dilutions) the rabbit is bled to death from the carotid artery, as much blood being obtained as possible, the serum separated, divided between small glass tubes of 2 or 3 cc. capacity, hermetically sealed, and preserved aseptically in the refrigerator. The hemolytic strength of the serum must be titrated before using.

**5. Suspension of Red Blood Cells.**—Blood is obtained from the

jugular vein of a sheep by means of an aspirating syringe. It is immediately placed in three or four times its volume of 0.85 per cent. sodium chloride solution containing 1.5 per cent. sodium citrate to prevent clotting. The corpuscles are removed from this solution by centrifugalization and further prepared by washing three times in five or six times their volume of 0.85 per cent. salt solution, and are finally brought to a 5 per cent. suspension in salt solution.

Before the syphilitic antigen, referred to hereafter merely as antigen, and the rabbits' immune serum, referred to hereafter as hemolytic amboceptor, can be used, their strength must be ascertained. The hemolytic amboceptor should be titrated first and afterwards the antigen.

**Titration of the Hemolytic Amboceptor.**—Allow the serum to stand at room temperature until all the complement disappears; this may require three or four days. Prepare a series of dilutions of the serum, beginning say at 1-400 and proceeding as follows: 1-600, 1-800, 1-1000, and so on up to 1-2000 or higher, if necessary. Set up a series of small test-tubes, 1 x 8 cm. is a convenient size, and into each place 0.25 cc. of a serum dilution, beginning at the highest and running to the lowest dilution. Next add 0.25 cc. of a 1-10 dilution of fresh guinea-pig serum (complement) and then 0.25 cc. of a 5 per cent. suspension of washed sheep-corpuscles to each tube. Bring the total volume up to 1.25 cc. by the addition of 0.5 cc. salt solution. Place the tubes in a thermostat at 37.5° C. for two hours and then allow to stand in the refrigerator over night. The tubes in which all the corpuscles have been completely hemolyzed will show a clear ruby red fluid with no sediment. Those in which hemolysis has been only partial will show a clear red fluid, perhaps of lighter shade than the preceding, and a sediment composed of the undissolved corpuscles in the bottom of the tube. On shaking, these tubes become cloudy. Those tubes in which no hemolysis has occurred show a clear colorless supernatant fluid and the corpuscles all sedimented at the bottom of the tube.

The highest dilution in which complete solution of the cells has taken place is said to be the "titre" or strength of the serum. In the Wassermann reaction and in titrating the antigen the hemolytic serum is used in one-half the maximum dilution which gives complete hemolysis in the above test. Thus if the titre of the hemolytic serum is 1-2000 it will be used in the subsequent tests in a dilution of 1-1000.

**Titration of the Antigen.**—Having ascertained the strength of the hemolytic amboceptor, the antigen can now be titrated for its ability to fix complement. For this purpose one requires a known syphilitic serum and a known normal serum.



Two parallel series of tubes are set up and into each tube of one series 0.25 cc. of a 1-5 dilution of syphilitic serum is placed and into each tube of the other series 0.25 cc. of a 1-5 dilution of normal serum.

A series of dilutions of the alcoholic extract of antigen is prepared, using 0.85 per cent. salt solution as the diluent. These dilutions may begin at 1-2 and run 1-4, 1-6, 1-8, and so on, up to 1-20 or higher if necessary. Into the first tube of each series 0.25 cc. of the lowest dilution of antigen is placed; into the second tube of each series the next dilution and thus throughout the series.

To each tube in both series is now added 0.25 cc. of a 1-10 dilution of guinea-pig serum (complement). All the tubes are now incubated at 37.5° C. for one hour, and then are added 0.25 cc. of hemolytic amboceptor in one-half the highest dilution which was found to cause complete hemolysis and 0.25 cc. of a 5 per cent. suspension of washed sheep-corpuscles. The tubes are then allowed to stand in the incubator at 37.5° C. for two hours and in the refrigerator over night, after which the readings are made.

If the antigen is efficient no hemolysis will have occurred in any of the tubes in the series in which syphilitic serum was used except those containing the higher dilutions of antigen, whereas in the series of tubes containing normal serum hemolysis will have occurred in all the tubes except perhaps those containing the lower dilutions of antigen. Thus, for example, it may happen that in the series containing syphilitic serum hemolysis does not begin until a dilution of antigen of 1-20 is reached, whereas in the series containing normal serum hemolysis is complete in all the tubes after a dilution of 1-4 is reached. This inhibition of hemolysis in the tubes containing the low dilutions of antigen in the non-syphilitic series is ascribed to an anti-complementary power of the antigen when used in excess.

Thus in the example just given antigen is available for use in dilutions which range between 1-4 and 1-20. In lower dilutions than 1-4 hemolysis is inhibited in the presence of normal serum and in higher dilutions than 1-20 hemolysis takes place even in the presence of a syphilitic serum. In the Wassermann reaction one uses antigen in double the lowest dilution which gives complete hemolysis in the series of tubes containing normal serum. Thus in the example chosen, above a 1-4 dilution of antigen was the lowest dilution which gave complete hemolysis in the presence of normal serum, therefore such an antigen would be used in a dilution of 1-8 in the Wassermann test.

Having prepared and titrated the antigen and hemolytic amboceptor and having obtained a known syphilitic serum, a known normal serum, serum from patients to be tested, and having inactivated all



Two parallel series of tubes are set up and into each tube of one series 0.25 cc. of a 1-5 dilution of syphilitic serum is placed and into each tube of the other series 0.25 cc. of a 1-5 dilution of normal serum.

A series of dilutions of the alcoholic extract of antigen is prepared, using 0.85 per cent. salt solution as the diluent. These dilutions may begin at 1-2 and run 1-4, 1-6, 1-8, and so on, up to 1-20 or higher if necessary. Into the first tube of each series 0.25 cc. of the lowest dilution of antigen is placed; into the second tube of each series the next dilution and thus throughout the series.

To each tube in both series is now added 0.25 cc. of a 1-10 dilution of guinea-pig serum (complement). All the tubes are now incubated at 37.5° C. for one hour, and then are added 0.25 cc. of hemolytic amboceptor in one-half the highest dilution which was found to cause complete hemolysis and 0.25 cc. of a 5 per cent. suspension of washed sheep-corpuscles. The tubes are then allowed to stand in the incubator at 37.5° C. for two hours and in the refrigerator over night, after which the readings are made.

If the antigen is efficient no hemolysis will have occurred in any of the tubes in the series in which syphilitic serum was used except those containing the higher dilutions of antigen, whereas in the series of tubes containing normal serum hemolysis will have occurred in all the tubes except perhaps those containing the lower dilutions of antigen. Thus, for example, it may happen that in the series containing syphilitic serum hemolysis does not begin until a dilution of antigen of 1-20 is reached, whereas in the series containing normal serum hemolysis is complete in all the tubes after a dilution of 1-4 is reached. This inhibition of hemolysis in the tubes containing the low dilutions of antigen in the non-syphilitic series is ascribed to an anti-complementary power of the antigen when used in excess.

Thus in the example just given antigen is available for use in dilutions which range between 1-4 and 1-20. In lower dilutions than 1-4 hemolysis is inhibited in the presence of normal serum and in higher dilutions than 1-20 hemolysis takes place even in the presence of a syphilitic serum. In the Wassermann reaction one uses antigen in double the lowest dilution which gives complete hemolysis in the series of tubes containing normal serum. Thus in the example chosen, above a 1-4 dilution of antigen was the lowest dilution which gave complete hemolysis in the presence of normal serum, therefore such an antigen would be used in a dilution of 1-8 in the Wassermann test.

Having prepared and titrated the antigen and hemolytic amboceptor and having obtained a known syphilitic serum, a known normal serum, serum from patients to be tested, and having inactivated all

supernatant fluid. In the tubes in which hemolysis has been complete the fluid will be a clear ruby red with no sediment. There may be all grades of hemolysis between these two extremes.

A negative reaction requires that there be complete, or almost complete, hemolysis, and in order that a reaction be called positive there should be no hemolysis, indicating complete fixation of complement. The sera which give partial hemolysis can be regarded only as doubtful positive or doubtful negative, according as the degree of hemolysis is small or great.

Certain controls are necessary, as indicated in the protocol. Tubes 2, 4, and 6 are controls to show that the sera, without the addition of antigen, do not give fixation in double the amount used in the test. Tube 11 shows that the antigen alone in double the amount used does not give fixation. Tubes 12 and 13 show that the hemolytic system (the term applied to corpuscles, hemolytic amboceptor and complement) is active not only with the dose of hemolytic amboceptor used in the tests, but with half that dose. Tube 14 is a control to show that the corpuscles used do not undergo hemolysis due to any extraneous or accidental cause.

Other controls might be introduced to show that neither the hemolytic amboceptor nor the antigen alone is capable of hemolyzing the corpuscles, but this was done when these two reagents were titrated and need not be repeated each time a set of tests is performed. In the beginning it is perhaps well to test the patient's serum to show that all the complement which it contained has been destroyed by heating it to 57° C. for 30 minutes. This is done by adding to 0.25 cc. of the inactivated patient's serum 0.25 cc. each of hemolytic amboceptor and corpuscle suspension and 0.5 cc. salt solution and observing that hemolysis does not take place.

After one has had considerable experience with the reaction it is not essential that a known positive and known negative serum be included in each series, but at first it is well to have these controls.

#### THE NOGUCHI MODIFICATION.

Noguchi recognized the value of the Wassermann reaction, but called attention to the fact that human serum contains a variable amount of natural hemolytic amboceptors for the corpuscles of a number of animal species, including the sheep. He pointed out that sera containing an excessive amount of natural hemolytic amboceptor for sheep-corpuscles might give complete hemolysis (a negative Wassermann reaction) even though they contained syphilitic amboceptors, thus leading to an error in diagnosis.

To overcome this difficulty and to simplify the test Noguchi introduced several modifications, the most important of which con-

sisted in substituting an antihuman hemolytic system for the anti-sheep system.

For a complete account of Noguchi's system the reader is referred to the original papers. The various modifications will be merely indicated here and but one of his methods of performing the test given.

**Antigen.**—May be used in liquid form or dried on paper. If used with inactivated patient-serum one may employ aqueous or alcoholic extracts, or pure acetone-insoluble lipoids. If used with active patient-serum only the pure lipoids can be used.

**Patient's Serum.**—May be used in active or inactive form; four times as much of the latter as of the former being necessary.

**Corpuscles.**—One per cent. suspension of human red blood corpuscles.

**Hemolytic Amboceptor.**—Serum of rabbit immunized against human erythrocytes in liquid or dried form.

**Complement.**—That contained in patient's serum if used in the fresh state, otherwise guinea-pig serum, fresh or dried on paper.

**Preparation of the Antigen.**—"Extract a mashed paste of liver, heart or kidney of man, ox, guinea-pig, rabbit, or dog with 10 parts of absolute alcohol at 37° C. for several days. Filter through paper and collect the filtrate. The latter is then brought to dryness by evaporation with the aid of an electric fan. The residue is then taken up with a small quantity of ether and five volumes of acetone added. A precipitate forms which is allowed to settle to the bottom of the vessel, and the supernatant fluid decanted off. We thus obtain a dark brown sticky mass. This insoluble residue contains antigenic lipoids and the strength must be estimated."

"Weigh out 0.2 gram of the sticky mass just mentioned and dissolve it in about 5 cc. of ether, then add gradually 100 cc. of physiological salt solution to make an emulsion. If flocculent it may be filtered through paper to remove the precipitate."

This solution of the antigen is then titrated in a way analogous to that described for Wassermann's method, except that the antihuman hemolytic system is employed instead of the antisheep system.

Noguchi uses antigen in the largest amount which is found to give no inhibition of hemolysis in the presence of normal serum, instead of half that amount as in the case of the Wassermann method. He recommends that the antigen be preserved in dried state and for this purpose he dissolves the acetone precipitate in ether (0.4 gram precipitate in 20 cc. ether) and saturates sheets of filter paper in this solution, which are afterwards dried and must subsequently be again titrated in order to determine how much antigen is contained in a piece of paper of given size.

**Preparation of Serum to be Tested.**—Two cubic centimetres of blood are required and this the author obtains by puncture of the finger and collection in small glass capsules, as described in the preceding section on "Opsonins." Inactivation of the serum is accomplished by heating to 55° C. for 30 minutes.

**Complement.**—Guinea-pig serum is used for complement. This may be preserved in dried form on blotting paper, but the author now recommends that fresh complement be used whenever available.

**Preparation of Hemolytic Amboceptor.**—Rabbits are immunized by intraperitoneal injection of washed human red blood corpuscles. The injections are given at four or five-day intervals. A total of five injections are given, progressing as follows: 5 cc., 8 cc., 12 cc., 15 cc., 20 cc. The rabbit is bled to death nine or ten days after the final injection and the serum saved.

The hemolytic strength of the serum is tested in a manner analogous to that described under the Wassermann method and is used in the fixation test in twice the smallest amount found to give complete hemolysis.

Tube number	Patient's serum		Complement 1-1½ dilution (40%)	Antigen amount determined by previous titration	Human R. B. C. 1% suspension	Incubate at 37.5° C. for 1 hour.	Hemolytic amboceptor amount determined by previous titration	Incubate at 37.5° C. 2 h.; allow to stand room temp. few h.	Resulting hemolysis
	Name	Capillary drop, about 0.02 cc.							
1	X	1 drop	cc. 0.1	1 unit	cc. 1	Incubate at 37.5° C. for 1 hour.	2 units	In tubes 1, 3, and 5 hemolysis will be complete or absent according as the reaction is negative or positive. Hemolysis should be complete in tubes 2, 4, and 6, showing that the serum alone does not fix complement.	
2		1 drop	0.1		1		2 units		
3	Y	1 drop	0.1	1 unit	1		2 units		
4		1 drop	0.1		1		2 units		
5	Z	1 drop	0.1	1 unit	1		2 units		
6		1 drop	0.1		1		2 units		
7	A known positive	1 drop	0.1	1 unit	1	Incubate at 37.5° C. 2 h.; allow to stand room temp. few h.	2 units	Absent.	
8	B known negative	1 drop	0.1		1		2 units	Complete.	
9		1 drop	0.1	1 unit	1		2 units	Complete.	
10			0.1		1		2 units	Complete.	

Noguchi recommends that the hemolytic amboceptor be preserved in dried state on filter paper and where this is done it has to be titrated again after it is dried on paper.

**Preparation of Corpuscles.**—A few drops of human blood are obtained from the finger and received directly into salt solution (0.9 per cent. sodium chloride solution is used throughout the test by Noguchi) washed and brought to a 1 per cent. suspension.

Having made the foregoing preparations the test may be set up. Two tubes are required for each serum tested and four control tubes are required, two for a known positive serum and two for a known negative serum.

The accompanying protocol indicates sufficiently well the various steps in the procedure.

#### THE VALUE OF THE FIXATION OF COMPLEMENT TEST IN THE DIAGNOSIS OF SYPHILIS AND PARASYPHILIDES.

Noguchi has collected the results of a number of investigators who have used the Wassermann reaction in the diagnosis of syphilis. The figures obtained by him are briefly summarized here:

Condition	Number of cases	Percentage positive
Primary syphilis.....	416	69.8
Secondary syphilis manifest.....	1605	89.4
Tertiary syphilis manifest.....	581	78.1
Early latent syphilis.....	1233	51.
Late latent syphilis.....	861	47.
Hereditary syphilis.....	125	94.5
Cerebrospinal syphilis.....	64	47.6
General paralysis.....	498	88.1
Tabes.....	216	62.66

Noguchi reports a higher percentage of positive reactions in syphilis and the parasyphilides with his method of performing the test than with Wassermann's method. One of the principal difficulties which has been experienced with Noguchi's modification in the hands of other men has been the difficulty of obtaining an efficient antihuman hemolytic system.

Positive reactions may occur in a few conditions where syphilis can be excluded with reasonable certainty, the most important of which are scarlet fever and leprosy. In other conditions reported in the literature positive reactions occur so infrequently as to be practically negligible.

Treatment with mercury or salvarsan may quickly cause the serum of a syphilitic patient to lose the power of giving a positive Wassermann reaction, so that for diagnostic purposes it is necessary to take this fact into consideration.

In cases suspected of tabes it is advisable to test both the serum and spinal fluid, since one and not the other may prove positive.

## CHAPTER VI

### EXAMINATION OF VARIOUS FLUIDS

AMONG these are the plasma, serum, lymph, the cerebrospinal, the various transudates and exudates, the cystic fluids, the synovial and amniotic fluids.

**SPECIFIC GRAVITY.**—This may be determined with an accurate aërometer (see page 95). Our figures given in the following pages were determined gravimetrically.

**DRIED CONSTITUENTS.**—In a weighed glass dish with a ground-glass stopper are weighed or measured from 10 to 30 cc. of the fluid in question. This is evaporated over a water-bath and then in vacuo over sulphuric acid. It is then dried at about 110° to constant weight, no higher if urea or other fragile bodies, as is usually the case, are present.

**PROTEIDS.**—In all may occur serum albumin, serum globulin, in some fibrinogen; the albumoses are rare. True peptone is said never to occur, while the glyco-proteids and the phospho-proteids occur in some; *e.g.*, the cystic fluids.

For examination of the proteids it is first necessary to remove the organized structures. This may be done by sedimentation, centrifugalization, filtration through paper or Kieselguhr. If fibrin is present, it will be evident to the naked eye, and shown positively by the rapid solution of the clot in artificial gastric juice, and its glassy swelling on the addition of 0.1 per cent. hydrochloric acid.

**ALBUMIN, GLOBULIN, FIBRINOGEN.**—From 20 to 50 cc. are mixed with an equal amount of saturated  $(\text{NH}_4)_2\text{SO}_4$  and allowed to stand one hour. The amount chosen should not contain more than 0.2 to 0.3 gm. of proteid for each precipitate. It is then filtered through a weighed filter, the precipitate washed with half-saturated  $(\text{NH}_4)_2\text{SO}_4$  until the filtrate gives no cloud with acetic acid and  $\text{K}_4\text{FeCN}_6$ .

(a) The filtrate is boiled, acetic acid added until faintly acid, it then boiled again, and filtered through a weighed ashless filter. The precipitate is washed with hot water, then with alcohol, then with ether, and brought to a constant weight at 120° C. It is then ashed and the weight of this subtracted to give the weight of the albumin.

(b) The precipitate on the filter paper is heated to 110°, washed with hot water, then alcohol and ether, dried to constant weight, and its ash subtracted. This will equal the weight of the serum globulin and fibrinogen.

For the determination of both together, see page 661.

**GLYCO-PROTEIDS and PHOSPHORUS-CONTAINING PRÔTEIDS.** (1) *Mucin.*—In general 100 cc. of fluid, diluted if necessary with water, are precipitated with acetic acid, filtered, and the precipitate washed with water acidulated with acetic acid. The precipitate is then dissolved in weak alkaline water, and reprecipitated with acetic acid.

*A. Mucin. Mucoid.*—A part of this precipitate is boiled on the water-bath with dilute mineral acid (HCl) and filtered, and the filtrate tested for sugar. The reduction of the copper is not as ready as by pure glucose solutions, hence considerable boiling may be necessary, and the reduced copper seen only after the fluid is cold and the precipitate settled.

*Mucin* is a glyco-proteid of a stringy consistency, insoluble in acetic acid even in excess. *Mucoid* is similar in nature, but differs in some physical characteristics. A sharp line cannot be drawn (see page 221).

*B. Phosphorus-Containing Proteid.*—A part of the precipitate is examined for organic phosphorus. It is ashed, the ash dissolved in dilute  $\text{HNO}_3$ , heated to boiling, concentrated somewhat, and then ammonium molybdate added in excess. A yellow color, and then a yellow precipitate which forms most readily at 40° C.,

is evidence of phosphoric acid. Or the precipitate may be dissolved in HCl, made strongly alkaline with ammonia, and then magnesium mixture added. The white precipitate of  $\text{NH}_4\text{MgPO}_4$  indicates phosphoric acid.

If the reaction for phosphorus be merely faint the test has no meaning, since all of the phosphates and lecithin cannot be washed from the mucin precipitate.

If organic phosphorus has been found, a part of the original precipitate is dissolved in NaOH, then HCl added, boiled to clear solution, supersaturated with ammonia and then precipitated with  $\text{AgNO}_3$ . A flocculent cloud indicates a nucleo-proteid, the silver salt of the nuclein base being precipitated. If none forms, the phosphorus body is a paranucleo-proteid.

**FAT, LECITHIN AND CHOLESTERIN.**—To from 20 to 50 cc. of the fluid, weighed or measured, are added from 3 to 4 volumes of absolute alcohol. This is allowed to stand until the next day with repeated stirrings, then filtered, the precipitate washed with absolute alcohol, and the precipitate placed in the cylinder of a Soxhlet ether-extraction apparatus. The alcohol filtrate is neutralized and evaporated at  $60^\circ \text{C}$ . The residue is taken up with alcohol and ether and re-evaporated. The residue is then taken up with ether and placed in the flask of this same Soxhlet apparatus. The precipitate is then extracted for hours. The ether extract is evaporated, the residue taken up in water-free ether, the filtered solution evaporated in a weighed beaker, and dried in vacuo over sulphuric acid to constant weight. This will be the combined weight of the fat, lecithin, and cholesterol.

This residue is dissolved in alcohol, alcoholic KOH added, and it warmed on the water-bath for one hour, then evaporated to dryness. The fat is now soap and glycerin. To the residue is now added water (not too little) and this shaken out several times with equal volumes of ether.

The ethereal extract is distilled to small volume, then evaporated in a weighed beaker to dryness. The residue contains soap and cholesterol. The former may be washed out with cold alcohol (small portions) slightly acidulated with HCl. The cholesterol left is dried at  $80^\circ \text{C}$ . and weighed.

The alcohol washings with the soap are added to the water extract of the previous separation, which now contains all the lecithin-phosphorus. This fluid is evaporated, the residue ashed, and the phosphorus determined.

(Distearyllecithin contains 3.84 per cent. of phosphorus, dipalmityllecithin 4.12 per cent.)

**LEUCIN. TYROSIN.**—The fluid is examined as fresh as possible. All of the albumin is removed by heat and acetic acid, or by precipitation with from three to four volumes of alcohol, heating on the water-bath, cooling, and filtering. The alcohol is removed by evaporation. The filtrate is precipitated with neutral, then with basic, lead acetate, avoiding carefully any excess, and filtered. The lead is removed from the filtrate, with  $\text{H}_2\text{S}$ , the filtrate evaporated, and examined for crystals (see page 259).

**SUCCINIC ACID,  $\text{CH}_2\text{COOH}.\text{CH}_2\text{COOH}$ .**—This acid occurs in many animal fluids in traces; sometimes in the fluid of hydrocephalus and hydrocele, much in echinococcus cysts, and in wool-fat. It is formed by the bacterial decomposition of proteids and sugar. It frequently occurs in acid milk in the intestine, in putrid pus, and in the alcoholic fermentation of sugar.

The fluid is freed of albumin by heat plus acetic acid. If it be urine which is tested, the albumin is first removed, the urine perfectly precipitated with baryta water, and the excess of this removed by  $\text{H}_2\text{SO}_4$ . The filtrate is evaporated to a residue, acidified with HCl, and extracted repeatedly with ether. The ether is evaporated off, the residue taken up with a small amount of water, and allowed to stand until crystallization. Or, the watery solution may be heated to boiling, nitric acid added drop by drop until it takes a slight yellow color, then evaporated. If no crystals form a portion of the residue is fused in a test-tube with ammonia



and zinc dust. If a match-stick wet with strong sulphuric acid is held at the mouth of the tube, the red color of pyrrol indicates succinic acid. In this test, however, hæmin and the indol derivatives must be excluded. These latter will give the reaction on heating alone. Hæmin on heating with zinc dust alone.

**LACTIC ACID,  $C_3H_6O_3$ .**—Of the three modifications of lactic acid the inactive, or lactic acid of fermentation, occurs oftenest in the stomach and intestine of man. The dextrorotatory form, or sarcolactic acid, occurs in the muscles, blood, pericardial fluid, aqueous humor, and intestinal contents. It occurs also in the urine in acute yellow atrophy and phosphorus poisoning, liver cirrhosis after respiratory distress, severe exercise, and before death. It occurs in pathological transudates often in abundance, in the bones in osteomalacia, and in the sweat in puerperal fever. The lævorotatory form has never been found in the body.

The fluid to be examined is made, if necessary, faintly acid with dilute  $H_2SO_4$ , boiled and filtered to remove the albumin. Baryta water is added as long as a precipitate forms, and the excess of the barium removed with  $CO_2$ . The filtrate is evaporated to a thin syrup, without heating above  $70^\circ$ , in order to avoid the brown color. Absolute alcohol to about ten volumes or more in amount is then added slowly to the syrup, it is well stirred, allowed to stand for some time, then poured off. The residue is dissolved in a little water. The procedure is repeated with alcohol once more, continuing in the same way. The alcoholic solution is poured off, filtered, the alcohol distilled off to a thin syrup, and the residue digested on the water-bath at a moderate temperature to drive off the alcohol. It is then cooled. To the thin syrup is added an equal amount of dilute phosphoric acid; it is brought into a large flask and shaken out with a large amount of ether which gradually takes up the lactic acid. The ether must be frequently renewed. The united ether extracts are then filtered clear, the ether distilled off, the residue dissolved in water, boiled for some time with an excess of  $ZnCO_3$ , filtered, washed with hot water, evaporated to a small volume on the water-bath, and allowed to stand until the zinc salt of lactic acid crystallizes out. Alcohol is then added to the mother liquid, and it allowed to stand longer, and another mass of crystals is obtained. The zinc salt is dissolved in hot water and the zinc precipitated by  $H_2S$ . The filtrate is then evaporated to a syrup containing the lactic acid.

It is wholly untrustworthy to attempt to recognize lactic acid simply from its crystalline form or by Uffelmann's test alone.

**INOSITE  $C_6H_6(OH)_6$ .**—This occurs in the urine of diabetics, in albuminuria, traces perhaps in each normal urine, but especially in polyuria, and in the echinococcus cysts.

The albumin is removed by heat, the phosphates precipitated by baryta water, the filtrate evaporated, and the creatinin allowed to crystallize out by boiling with from one to four volumes of alcohol. If a heavy precipitate results which sticks to the glass, the fluid is simply decanted, but if flocculent, it is filtered through a heated filter and then allowed to cool. The fluid then stands for twenty-four hours. If inosite is present, crystals will form which may be filtered out and washed with cold alcohol. The alcohol precipitate may be dissolved in boiling water, from three to four volumes of hot alcohol added, and the above procedure repeated to recover the inosite therein contained. If no crystals form, to the clear alcoholic filtrate is added little by little ether until a slight milky cloudiness results which does not disappear. This is then allowed to stand for twenty-four hours. All the inosite is precipitated as mother-of-pearl plates.



In case the urine is examined, it is first precipitated by baryta water and the filtrate, after heating, precipitated with PbAc, avoiding an excess. It is allowed to stand, is filtered, the precipitate washed, suspended in water, decomposed by  $\text{H}_2\text{S}$ , filtered, the filtrate evaporated. One then proceeds as above.

Inosite crystallizes in rhombohedral crystals which melt at  $225^\circ \text{C}$ ., are soluble in water, 1:75, insoluble in alcohol or ether. It does not ferment, nor does it rotate the plane of polarization; it dissolves  $\text{Cu}(\text{OH})_2$  without reduction, is precipitated by PbAc, and does not give crystals with phenylhydrazine.

**SCHERER'S TEST.**—A small amount of the precipitate of the crystals is evaporated with nitric acid on a platinum-foil almost to dryness. To the residue are added ammonia and one drop of  $\text{CaCl}_2$  and the evaporation continued to dryness. A beautiful rose color results. The crystals must be pretty pure to give a positive test.

**SEIDEL'S TEST.**—This test is similar to the above with the exception that strontium acetate is used instead of calcium chloride, and a green color with a violet precipitate results. This test is positive if 0.3 mg. of inosite be present.

**ALLANTOIN,  $(\text{CO})_4(\text{NH})_3\text{NH}_2$ .**—This body is found in the urine of the new-born child. A slight trace is said to occur in all normal urines, and especially those of pregnant women. It occurs in some ascitic fluids, in liver cirrhosis, and in certain ovarian cysts.

The albumin is removed by heat and acetic acid. The fluid is then precipitated with  $\text{HgNO}_3$ , the precipitate washed, suspended in water, decomposed with  $\text{H}_2\text{S}$ , and filtered. To the filtrate a little ammonia is added, and the whole evaporated to a small volume on the water-bath. The clear fluid is then precipitated with ammoniacal  $\text{AgNO}_3$ . (The precipitate is soluble in excess of ammonia, which must be avoided.) This is allowed to stand, the silver salt of allantoin is collected on the filter, washed, suspended in water, decomposed with  $\text{H}_2\text{S}$ , the filtrate evaporated, and allowed to crystallize.

The Loewy method is recommended for the urine. The faintly acid urine is precipitated with mercurous nitrate (which is dissolved in as little acid as possible plus some metallic mercury), filtered, the precipitate well washed, the filtrate is then precipitated with  $\text{H}_2\text{S}$ , and filtered, the filtrate warmed to drive off this gas,  $\text{MgO}$  then added, and the whole precipitated with  $\text{AgNO}_3$ . This precipitate is filtered, washed, suspended in water, and decomposed with  $\text{H}_2\text{S}$  while warm; the filtrate evaporated to dryness, the residue extracted with hot water, and when cold precipitated with  $\text{Hg}(\text{NO}_3)_2$ . The precipitate is well washed, decomposed with  $\text{H}_2\text{S}$ , the filtrate evaporated to a concentrated solution, whereupon the allantoin will crystallize out in glistening prisms, which are odorless, tasteless, soluble in 160 parts of cold water, and more in warm, insoluble in absolute alcohol or ether. For its identification the silver salts are studied, concentrated solutions being precipitated by ammoniacal  $\text{AgNO}_3$ . This precipitate is soluble in excess of ammonia. The white flocculent precipitate on standing becomes granular. If dried at  $100^\circ$  it gives an easy reduction of silver. The silver salt of the allantoin dried in vacuo gives on fusion 40.71 per cent. Ag.

**Quantitative Analysis of Serous Fluids.**—The method given by Thierfelder<sup>1</sup> is the one we use and of which we here give an outline. We have tried it on all sorts of fluids, and while each step is satisfactory, the routine, as a whole, is not, since fluids vary greatly in their composition, and the method hits none exactly. If one uses enough of some fluids to obtain a measurable amount of certain constituents, the amount of other constituents may be in such vast amounts as to render the whole impossible; determine these latter with a workable amount, and the former are an almost vanishing quantity. Hence it is better to determine each substance or group in a special portion. We give the outline of the analysis.

To from 20 to 50 cc. of the fluid measured or weighed, and freed from the

<sup>1</sup> Hoppe Seyler, *Chemische Analyse*, 1903.

formed elements by filtration, are added from three to four volumes of absolute alcohol. This is allowed to stand a few hours, filtered through a weighed filter, and washed a little with alcohol. This will be Filtrate I. The precipitate is then washed with boiling alcohol, into the same flask with ether, and then again with alcohol; these combined are Filtrate II. The precipitate is then washed with boiling water thoroughly, Filtrate III. The above precipitate will contain the proteid and a few salts, hæmoglobin if any be present, but not much of the other pigments. It is now washed once in alcohol and dried at  $120^{\circ}$  to constant weight, after the precipitate next to be mentioned has been added to it. It is then ashed and the weight of the ash subtracted from that of the precipitate, giving the weight of the *proteids*.

Filtrate I. is evaporated at a temperature not above  $60^{\circ}$  C. To this residue is then added Filtrate II. The residue and fluid are well mixed and the fluid decanted through a dried and weighed paper. It is then washed repeatedly with absolute alcohol, then with ether, and decanted each time, but none of the precipitate is allowed to get onto the paper (B 3). Onto the residue is now poured Filtrate III., the residue well mixed, and then all filtered through the same filter-paper, but into another flask from that which has received the above-mentioned decanted fluids. The precipitate is now brought on the paper and washed with water. This precipitate is dried and added to the above-mentioned proteid precipitate, since it contains that amount of proteid which was lost in the first precipitation. The watery extract of the above is evaporated in a small weighed porcelain dish in a water-bath, dried at  $110^{\circ}$  to  $115^{\circ}$  C. to constant weight, and weighed.

B. 2. It is burned at a moderate heat, ashed, and the weight of the ash determined.

B. 3. The alcohol-ether extract is evaporated on the water-bath at a temperature not above  $60^{\circ}$  C., dried in vacuo over  $\text{H}_2\text{SO}_4$ . To the residue is added ether, and filtered into a flask through a small paper, washing repeatedly with ether. This will contain the urea, sugar, soaps, sodium chloride, fat, lecithin, cholesterin, and the cholesterin ester.

B. 3 *a* is the residue of the above. This is washed from the beaker and paper into a small weighed porcelain dish, evaporated, dried at a temperature from  $110^{\circ}$  to  $115^{\circ}$ , fused at a moderate heat, and weighed.

B. 3 *b*. The ethereal extract is treated as on page 693. It contains fat, cholesterin, lecithin, cholin, etc.

#### CEREBROSPINAL FLUID

The *normal amount* of this fluid is said to vary from 60 to 80 cc.; normally from 5 to 10 cc. or more may be obtained by puncture. It is relatively most abundant in the first years of life. It is pathologically increased in certain infectious diseases, in meningitis, and always in hydrocephalus and in general paralysis of the insane. Coriat was able to obtain in alcoholic cases from 10 to 100 cc., in dementia præcox even 50 cc., and in general paralysis, sometimes over 100 cc. In senile cases one gets even 60 cc.

In *color* the fluid is either absolutely limpid or has a slight yellowish color due to lutein, the pigment of blood-serum. In subdural hemorrhage the fluid may be red, in jaundice a greenish-yellow, while the presence of pus will of course give it an opaque yellow color. It is also stained by certain drugs, as, for instance, methylene blue.

In *reaction* it is normally alkaline, but after death it rapidly becomes acid. Its reaction varies much, and it is said sometimes even to be acid during life, due to lactic acid fermentation. But in as short a time as ten minutes after death it has been found acid; as in one

case after epileptiform convulsions, in which case the (inactive) lactic acid was said to have been present. Its reaction depends directly upon the reaction of the brain-tissue.

The *specific gravity*, Coriat states, is from 1007 to 1010 normally (Halliburton, 1006 to 1008). In various diseases this varies much, and nothing specific has been determined. In general paralysis 1009 to 1012 are common figures; in hydrocephalus 1008 to 1009, thus within the normal limits.

In the cerebrospinal fluid is found a *reducing body*, 0.04 to 0.05 per cent., the nature of which has been much disputed. Halliburton claims that this is similar to pyrocatechin, that it is not sugar, is always present, and is increased by repeated tapplings. This reducing body reduces copper but not bismuth, does not ferment, is optically inactive, and gives no osazon with phenylhydrazine. Mott and Halliburton found this body absent in twelve of fourteen cases of general paresis; it is absent in tuberculous, and especially epidemic, cerebrospinal meningitis. Others claim that glucose is present in hydrocephalus (Cavazzini); in diabetes (Schaefer), in which case 0.32 to 0.35 per cent. is said to have been found; in grave pneumonia it is increased. Coriat thinks that the body is glucose, or, at least, is not pyrocatechin.

*Urea* is present but has no pathological significance. The normal amount is from 0.01 to 0.05 per cent. Much is present in the fluid of a case of hydrocephalus; much in nephritis, even 0.45 per cent., and considerable in that of arteriosclerosis.<sup>2</sup>

The *proteids* found are globulin, nucleo-proteid, and protalbumose. The total proteid according to Quinke is from 0.2 to 0.5, Ricker 0.5 to 1, and Gumprecht 0.25 per litre. Halliburton found globulin present in thirteen cases, and in six cases albumose also, while in three, two of which were clearly cases of inflammation, albumin was also found. No fibrinogen is ever found normally. (To detect this a little blood-serum is added to the cerebrospinal fluid, and its presence is evinced by fibrin formation.) Serum albumin is said to be normally absent. In meningocele albumose and peptone have been found.

In general paresis the total solids are even 2.39 p. m.; the proteids are considerably increased. There is some increase in hydrocephalus, in inflammatory conditions, in cases with stasis due to brain tumor (2 to 4 p. m.), after repeated tapplings, in apoplexy, and in meningitis (as a rule, 2 to 3 p. m.; but if purulent 7 to 9 p. m.).

Mott and Halliburton recommend the following quantitative method: The fluid is made acid with acetic acid, two volumes of absolute alcohol are added, it is then boiled, filtered, the precipitate dried at 110° C., and weighed.

<sup>2</sup> See Widai and Froin, *Gaz. des Hôp.*, No. 122, 1904.

In eight cases of general paresis the average percentage was 0.239. In two of spina bifida 0.088 per cent. In general paresis proteoses and peptones were absent, and globulin with a little nucleoproteid were present.

This latter is determined in one litre of the fluid to which alcohol has been added, and the precipitate digested in water. If the undissolved residue is found to contain a high percentage of phosphorus, indicating nuclein, the residue is washed with 0.2 per cent. HCl, heated on the water-bath at 100° C. with fuming HNO<sub>3</sub> and a small amount of H<sub>2</sub>SO<sub>4</sub> and KClO<sub>3</sub>. The residue is dissolved in HNO<sub>3</sub> and ammonium molybdate added; a yellowish crystalline precipitate results.

A very good idea of the amount of proteid present may be gained by the heat-acetic-acid test. A normal fluid remains clear on standing some hours. If it be boiled no cloud at all results, but on adding a drop of dilute acid a very faint white opalescence appears, which will separate in fine flocculi. If the fluid is mixed with an equal amount of saturated ammonium sulphate solution the globulin is precipitated. The clear filtrate is then acidified with acetic acid and boiled. The precipitate will be the albumin.

The saline constituents resemble those of other serous fluids.

The *toxicity* of the fluid has been found increased in general paresis, also after epileptic seizures. Its poisonous qualities are due to cholin and other products of nerve degeneration.

*Cholin* is a decomposition product of lecithin, the chief component of the myelin sheaths, and is found where there is nerve disintegration. It is a body which is soluble in water and alcohol, insoluble in ether, is precipitated by PtCl<sub>6</sub> as polymorphous crystals, which, however, if recrystallized from warmed 15 per cent. alcohol are regular octahedra. These crystals are insoluble in alcohol and ether, but soluble in water.

The careful technique given by Coriat is as follows: The proteids are first precipitated by 95 per cent. alcohol in excess, the filtrate evaporated over the water-bath at 40° to dryness, extracted with absolute alcohol, filtered again and evaporated to dryness. This process is repeated several times, the temperature always being kept low. All traces of proteid and potassium salts are thus removed. The final residue after extraction with absolute alcohol is a syrup of a light color, which is divided into two parts. The first is dissolved in distilled water, and the second in 15 per cent. alcohol. The watery solution is tested for proteid by the biuret, Millon, and other proteid reactions, which must all be negative; for cholin by the ordinary reactions for alkaloid (phosphotungstic and phosphomolybdic acids, *et al.*), which must all be positive. To the alcohol solution are then added four drops of 4 per cent. PtCl<sub>6</sub>, and this is evaporated in a watch-glass over CaCl<sub>2</sub>. For a positive test tannic acid should give no precipitate (thus neurin is excluded); phosphotungstic acid a white precipitate, phosphomolybdic a yellow, also PtCl<sub>6</sub> and AuCl<sub>3</sub>, and Lugol's a brown precipitate. On evaporating the 15 per cent. alcohol solution, large yellow octahedral crystals must be formed, easily soluble in water (therefore not neurin). Their size, solubility in water, and the fact that the watery solution gave the alkaloid reaction, excludes potassium. These crystals, if in a sufficient amount, may be dried and the platinum determined, which should be 34.8 per cent.

In the same hydrolysis with lecithin are formed the glycerophosphoric acids and stearic acid. The latter unites with the glycerol radicals to form the neutral fats upon which the Marchi stain depends.

The cholin is eliminated in the cerebrospinal fluid and the blood. Glycerophosphoric acid is eliminated in the urine.

The presence of cholin indicates nerve disintegration. It has been found, however, in a wide variety of nervous disturbances, general paresis, combined sclerosis, insular sclerosis, alcoholic neuritis, beriberi, senile dementia, delirium tremens, *et al.*, roughly in amount parallel to that of proteid present, both being a measure of nerve-tissue disintegration. Mott considers that it cannot be used to separate the organic from the functional disturbances unless the organic disturbance be active at the time the fluid is examined. Although it occurs in such a variety of cases, its most constant occurrence is in general paresis, in which disease Coriat found it present in all of fourteen cases, yet with no relation between amount and anatomical findings.

We add a table of a few analyses we have recently made, calling particular attention to the high solid content in stasis (due to brain tumor), and to the difference between the ventricular and spinal fluids in a case of hydrocephalus, a difference which we had noted in two previous cases.

The cerebrospinal fluids will not keep, in fact are full of bacteria in a few hours; hence must be used while fresh.

As regards cholin our experience is limited, but we fail to find the demonstration of octahedral crystals so very easy.

#### CEREBROSPINAL AND VENTRICULAR FLUIDS

No.	Case.	Amount.	Sp gr. (grav.)	Solids, per cent.	Proteids, per cent.	Salts and Extractives soluble in H <sub>2</sub> O; insoluble in Alcohol.	Total Ash, per cent.	Extractives and Salts soluble in Alcohol; Urea, Sugar, NaCl, etc.	Total Ash, per cent.	Soaps, Glycerin, } fat. Cholesterin, Cholin, etc., per cent.
1	Normal child.....	13	1007.4	.....	.....	.....	.....	.....	.....	.....
2	Normal child.....	22	1008.3	.....	.....	.....	.....	.....	.....	.....
3	Hydrocephalus : Cord .....	.....	1002.	.....	.....	.....	.....	.....	.....	.....
4	Brain (fluid from) Hernia of brain (tumor).....	50	1006.2	0.96	0.0991	0.2703	0.507	0.2937	.....	.....
	Later .....	630	1006.9	2.5132	0.1112	0.5964	0.5166	.....	.....	0.023
	Later..... (Ventricular fluids.)	450	1007.7	.....	.....	.....	.....	.....	.....	0.016
5	Tumor of brain.... (Hernia.)	200	1011.6	.....	.....	.....	.....	.....	.....	.....
6	Gunshot wound; head.....	25	1009.2	.....	2.664	0.7839	0.68	0.1988	.....	.....
7	Cerebrospinal meningitis.....	100	1007.	.....	.....	.....	.....	.....	.....	.....
8	Streptococcus meningitis.....	25	1009.2	.....	0.1628	.....	.....	.....	.....	.....
9	Tuberculous men- ingitis .....	.....	1018.8	.....	0.066	0.5629	0.4712	0.4112	0.2445	0.0185
10	Pneumonia; men- ingeal symptoms..	10	1006.	.....	.....	.....	.....	.....	.....	.....
11	Paresis?.....	30	1008.	.....	0.0597	.....	.....	.....	.....	.....

CLINICAL EXAMINATION OF CEREBROSPINAL FLUID.—During recent years this subject has received careful study and with very valuable results. The method of obtaining the fluid by lumbar puncture cannot here be described further than by adding that a long glass tube with barometer bore should be attached to the needle, and the height noted to which the fluid, before any is removed, will rise in this tube held vertically. This is a measure of the pressure in the subarachnoid space and normally varies from 120 to 180 mm. The amount to be removed will depend partly on the drop in pressure caused by the loss of fluid, but more on the provisional diagnosis. In any doubtful case a safe rule is never to remove over 5 cc. at a time, for in patients with brain tumor the fluid is often under high pressure, and sudden death has sometimes followed lumbar puncture. The cells of the fluid should be counted, the approximate amount of its protein noted, and, if acute meningitis is suspected, smears and cultures made. In order for the cells to be counted the perfectly fresh spinal fluid is mixed with a staining fluid (*e.g.*, Unna's polychrome methylene-blue) in a leucocyte counter. The tube, filled first to the 0.5 mark with the stain and then to the 11 mark with the spinal fluid, is shaken and allowed to stand a few minutes. The cells are counted, and the differential count made on a common ruled blood counting chamber, but better on a special (Fuchs and Rosenthal's) slide, the chamber of which is 0.2 mm. deep, and the sides of the square of which are 4 mm. long. The volume of fluid bounded by this square will be 3.4 cmm. If  $A$  = the average number of cells in the squares counted, then  $\frac{10A}{32}$  = number of cells in 1 ccm. of the diluted fluid. The correction of 5 per cent. may be made for the slight dilution. In normal fluids from 1 to 5 (some say as many as 7, others, even 10) cells per cmm. may be found.

In case the fluid cannot be examined while fresh, it may later be centrifugalized for 15 minutes, and the sediment may be spread on a slide into a round smear about 7 mm. in diameter, fixed, and stained. In normal fluid not over 4 lymphocytes will be found in each field of a 400 magnification.

For the determination of the protein see page 697.

If the fresh fluid is turbid, a purulent meningitis is present. Smears of fresh fluid should be stained, and cultures made, preferably on blood agar, in order to determine the infecting organism. Among the organisms most often present in such cases are, *Micrococcus intracellularis meningitidis*, *Diplococcus pneumoniae*, *Bacillus influenzae*, *Streptococcus pyogenes*, and several other organisms (*e.g.*, *Bacillus typhosus*, *Bacillus coli*, *Bacillus paratyphosus*, *Bacillus mallei*, *Bacillus pestis*, staphylococci, etc.).

*Micrococcus Intracellularis Meningitidis*.—This organism (see Figs. 122c and 122d) resembles the gonococcus in morphology. While it occurs also extracellular, its most typical appearance is as intracellular biscuit-shaped diplococci which vary noticeably in size. The meningococcus is Gram-negative, stains easily with the common bacterial stains, and can be grown on many of the common culture media.

It is usually taken for granted that the organism causing "septic influenzal meningitis" is *Bacillus influenzae*. Recently, however, this has been doubted, since the organism found has a different pathogenicity to animals than *Bacillus influenzae*.\*

In *tuberculous meningitis* the fluid is often very clear at first (even though it may contain 500 cells per cmm.), but on standing a reticulum of fibrin or a denser clot separates. To demonstrate tubercle bacilli, the fluid may be centrifugalized, either fresh or after the addition of an equal volume of alcohol to facilitate precipitation, or the tubercle bacilli may be "salted" to the upper layers of the fluid as in the sputum; but the best method is to allow the clot to separate and to make smears from this. If no organisms are found, a guinea-pig may be inoculated with some of the fluid. Without the demonstration of the tubercle bacillus tuberculous meningitis may be suspected when the fluid is found to be under increased pressure and there is a definite increase of its cellular elements and proteins. There is a more marked spinal leucocytosis in this condition than in tabes or paresis, since in this the count is usually above 100 cells (80 to 952) per cmm., while in the other two it is usually below 100 cells. The protein content is usually over 0.5 gm. per litre, while the pressure ranges from 240 to 550 mm., the average being 365 mm.†

The most interesting recent applications of the *cell count of spinal fluids* are in the diagnosis of cerebral lues, general paresis, and tabes dorsalis. In these diseases there are a chronic posterior meningitis and a slight, though usually definite, spinal leucocytosis. In general paresis an increased lymphocytosis and an increased amount of protein in the spinal fluid are the earliest symptoms. A negative find is often of more value than a positive one. In 80 cases the counts varied from 5 to 204 cells per cmm. In 9 of these the count was under 5, in 6 over 100 (Rous). In 25 cases of this disease Cornell found from 12 to 216 cells, the average being 52. Of these cells, from 45 to 97 per cent. were small lymphocytes, from 0 to 15 (average 4) per cent. large lymphocytes, from 1 to 56 (average 18) per cent. polymorphonuclear neutrophils, and from 0.1 to 5 (average 1.5) per cent. plasma cells.‡ In the diagnosis of cerebral lues the cell counts

\* Thursfield, The Quart. Jour. of Med., Oct., 1910, vol. iv, No. 13.

† Rous, Am. J. Med. Sci., April, 1907.

‡ Cornell, Am. Jour. Insan., July, 1907, vol. lxiv.



have thus far been of little aid. A slight spinal lymphocytosis has been found present in about half the cases of locomotor ataxia studied, and the same is true of some cases of multiple sclerosis. On the other hand, in a large group of mental and nervous diseases, among which are included the psychoses of arteriosclerosis, chronic alcoholism, chronic delusional states, dementia præcox, epilepsy, most maniacal

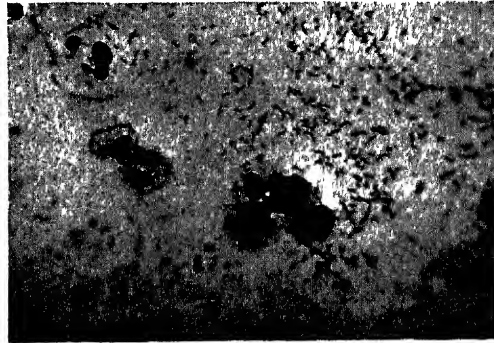


FIG. 122e.—Smear of the spinal fluid of a case of meningitis due to *Bacillus influenzae*.

and hypomaniacal conditions, the psychoneuroses, uræmia, hydrocephalus, and cerebral neoplasm, studies of the spinal fluids have thus far proved negative.

#### TRANSUDATES AND EXUDATES.

Although the pathologists believe that no sharp distinction should be attempted between exudates and transudates so far as the mechanism of their origin is concerned, yet the clinical chemist is forced to differentiate rather sharply between them on the basis of their physical and chemical properties. In some measure at least a transudate is less the result of an inflammation and more of a filtration than is an exudate, which is more the result of an inflammation. The transudates resemble lymph; they contain few formed elements and almost no fibrin. Their proteids are serum albumin, serum globulin, and a little fibrinogen, yet not enough of the last to coagulate these fluids spontaneously, although they will coagulate if blood be added. The exudates are richer in formed elements, coagulate spontaneously, contain the so-called "nucleo-albumin" and a mucoid substance. Some claim that fluids whose specific gravity is under 1018 are usually transudates; and those in which it is over 1018, exudates. The list of extractives which may be present in these fluids includes urea, glucose, creatinin, uric acid, lactic acid, inosite, succinic acid, allantoin; pathologically occur leucin, tyrosin, bile acids and pigments, fat, lecithin, and cholesterin.

This "nucleo-albumin," or better "euglobulin," is valuable in dis-



tinguishing these two classes of fluids. If a few drops of acetic acid be added to a clear exudate, a cloud of varying depth, but usually quite dense, will form. It is rather soluble in excess of the acid. The cloud in transudates is very much lighter.

**Peritoneal Fluid.**—In cachexia and hydræmia this fluid is slightly colored, of a milky opalescence, does not clot spontaneously, has a specific gravity of from 1005 to 1015, and almost no cells.

In chronic passive congestion the specific gravity is usually lower than 1020. Sometimes it contains 35 gms. per litre of proteid. In cases of cancer of the peritoneum the fluid is turbid with cells, of a dirty grayish appearance, a high specific gravity, and often clots spontaneously. The serous fluid present in inflammations is of a straw or lemon-yellow color, somewhat cloudy from the formed elements, coagulates spontaneously; proteid 30 gms. or more per litre, and a specific gravity of 1030 or above. Mucoid substance is perhaps always present.

This is proven by removing the albumin by heat and then by precipitating the filtrate by alcohol. A precipitate is formed from which one can split off a reducing body.

In the ascitic fluid may also be determined urea, uric acid, allantoin, xanthin, creatinin, cholesterin, and sugar.

The fluids we have examined had a specific gravity varying from 1005.5 to 1019.8, the solids from 1.3 to 4.5 gms. per litre, globulin, 40 to 50 per cent. None were very acute cases.

**Pleural Fluid.**—Physiologically, there is not enough pleural fluid present to be analyzed. The pathological fluid may vary from serous to sero-purulent to purulent or hemorrhagic. In hydrothorax the specific gravity is lower than 1015 as a rule, the albumin from 10 to 30 gms. and the fibrinogen hardly 0.1 per litre. In pleurisy the exudate has a specific gravity above 1020 as a rule, albumin 30 to 65, and the fibrinogen 1 per litre.

In nine recent cases the specific gravity of the fluids varied from 1012.2 to 1025.2, and the solids, from 3.12 to 7.926 per cent. The more acute the case the higher the figures. The amount of total proteid varied from 2.837 to 6.529 per cent., of which from 39 to 64 per cent. was globulin. The amount of globulin depended on the acuteness of the case. The acetic acid precipitate was markedly more in the acute inflammatory cases than in the transudates. It is interesting what a little difference the clotting makes. In a case of acute tuberculous pleurisy, before clotting the specific gravity was 1022.1 and the globulin 2.875 per cent.; after clotting (densely), the figures were 1021.7 and 2.376 respectively. To clear these fluids with the centrifuge is better than with Kieselguhr, since the extractions found are lower in the latter case.

THE CYTODIAGNOSIS of the serous fluids has been a fertile subject recently, all work being directed to one point, namely, the possibility of a diagnosis of tuberculous pleurisy or peritonitis based on a lym-

phocytosis. Other organisms cause a migration of the polymorphonuclear cells. Concerning the tubercle bacillus there are some who believe that the lymphocytosis is an active migration of the small mononuclear blood-cells; others that these cells are really derived from the endothelial lining of the serosa.<sup>5</sup> It is true that in some cases of so-called proliferative pleurisy or peritonitis there are found a great many free endothelial cells and large groups in mulberry-like masses in the fluid. It is also true that these endothelial cells undergo degenerative changes,

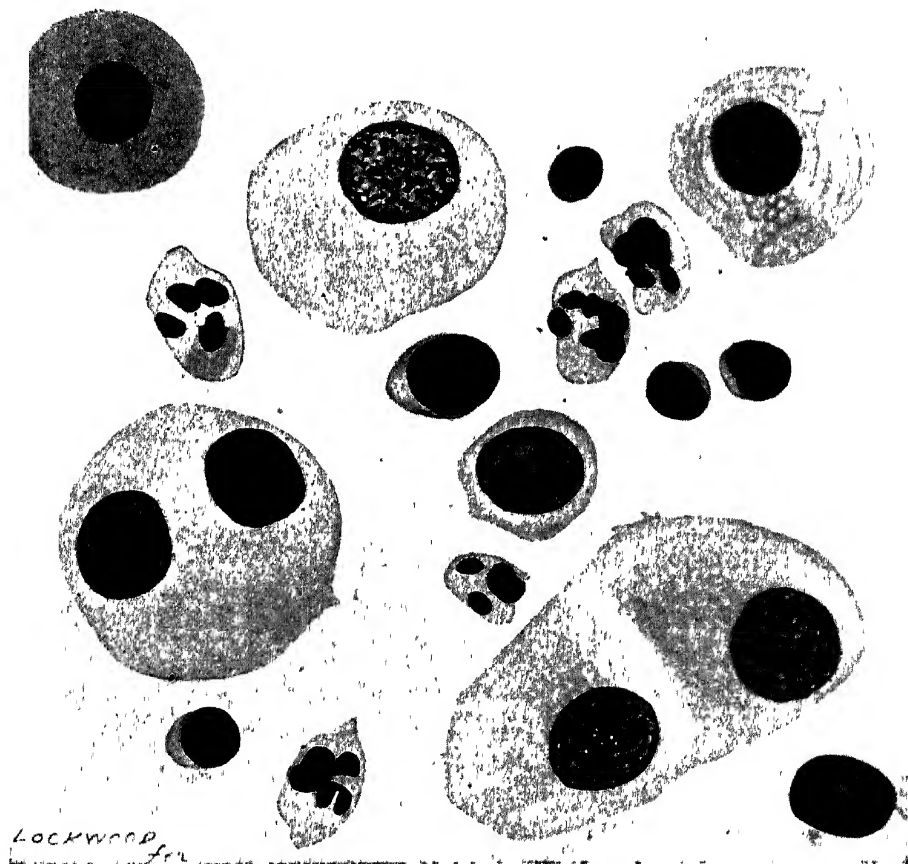


FIG. 123 —Cells from a pleural exudate, showing the transitions from large to small cells (lymphocytes).  $\times 900$ .

the result of which is a cell similar to a lymphocyte; but in tuberculous pleurisy the cells are chiefly typical lymphocytes and the stages of condensation of larger cells not so evident. In Fig. 123 we have drawn some of the cells from a chronic pleurisy, showing their transitions from very large flat to small compact cells. The lymphocytes in the picture seem different.

These fluids are best examined while fresh, the cells being centrifugalized before clotting occurs. Smears are made, and stained with a polychrome-methylene-

<sup>5</sup> See Patella, Deut. Arch. f. klin. Med., April 17, 1902.

blue-eosin mixture. That recommended by Musgrave is Wright's mixture diluted with methyl alcohol 3:1. This stain is left on one-half minute, then diluted with water for three minutes (see page 459).

In hydrothorax the cells are few and mainly endothelial; even in the serous stage of pneumococcus and streptococcus pleurisy there is a great preponderance of polymorphonuclears; in tuberculous inflammations the lymphocytes are the predominating cell, since the infection is weak and long-standing. This is as far as most observers go. Others go farther. Barège, for example, says that in the fluid of cardiac and Bright's disease, if the fluid contains many endothelial cells, few small mononuclears, and fewest polymorphonuclears, the origin is mechanical; a slight increase of the last cells means congestion of the lungs, and a considerable polynucleosis means lung infarct. The work of 1903 was in favor of cytodagnosis, but then the pendulum swung, and now workers are sceptical.<sup>6</sup>

A study of cases from this clinic was made by Bunting.<sup>7</sup>

The exceptions to such rules are too numerous. Some find lymphocytosis in transudates, in pneumococcus, influenzal, and staphylococcus infections; and polynucleosis when strictly there is no inflammation, as in infarcts, sunstroke, and cancer. Some called the findings "relative," others said "problematical." We use cytodagnosis very little now, since it is easier to find the organisms themselves, and if we find them we are certain.

In some cases Mastzellen are greatly increased. In tuberculous pleurisy there is a slight eosinophilia (2 to 5 per cent.), in acute pleurisy sometimes a high eosinophilia (of 10 to 74 per cent.) which means a good prognosis.

The possibility is always present of diagnosing cancer of the pleura or peritoneum by the cells of the fluid,<sup>8</sup> and especially by the small mass sometimes found sticking to the end of the needle.

INOSCOPY is the method proposed by Jousset<sup>9</sup> for isolating the tubercle bacilli from exudates. The fluids were allowed to coagulate spontaneously, or a little horse serum added which produced this result. The clot is digested, the resulting fluid centrifugalized, and smears made and stained for tubercle bacilli. The clot will have caught most of the organisms. It is first pressed out, then torn into fragments and mixed with a fluid consisting of pepsin, 1 to 2 gms., glycerin, 10 cc.; HCl 40 per cent., 15 cc.; NaF, 3 gms.; water, 1000 cc. Jousset claimed that digestion took but three or four hours. He obtained astonishingly good results, so good that his critics hint that he mistook masses of hæmoglobin or fuchsin and scratches on the glass, etc., for tubercle bacilli (Körmöczy and Jassinger).

The fluid may be centrifugalized while very fresh, or injected into a guinea-pig. We have not had good success with inoscopy.

<sup>6</sup> See especially Miller, *Am. Med.*, November 12, 1904.

<sup>7</sup> *J. H. H. Bull.*, July, 1903.

<sup>8</sup> *La Sem. Méd.*, No. 3, 1903.

<sup>9</sup> Steiner, *J. H. H. Bull.*, October, 1901.

**Pericardial Fluid.**—This fluid normally is of a lemon-yellow color, slightly viscid, and seems to contain more fibrin than other physiological fluids. The solids are from 37.5 to 44.9 gms. per litre; albumin, 22.8 to 24.7; soluble salts, from 8 to 9; insoluble salts, 0.15; extractives, 2 per litre.

The fluid of a recent case had a specific gravity of 1020.4; solids, 5.8 per cent.; total proteid, 3.91, and globulin only 15.2 per cent. of this.

**Synovial Membrane.**—The fluid is alkaline, thick, sticky, viscid, yellowish in color, cloudy often from the cell detritus, or clear. It contains albumin, salts, and a body which is physically like mucin, but which cannot be, since no reducing body can be split off. Neither is it nucleo-albumin. Salkowski has given it the name "synovin."

The fluid from a recent case of rheumatism, and which clotted firmly, had a total proteid content of 4.3 per cent.; water-soluble extractives, 1.07 (ash, 0.606) per cent.; alcohol-ether-soluble extractives, 0.076 (ash, 0.046) per cent.; fat fraction, 0.35 per cent.

**Chylous Fluids.**—Fluids which are milky in appearance have always attracted considerable attention. Sometimes a fluid may be truly chylous, in which case from 3.86 to 10.3 per litre of fat are often obtained, and in Minkowski's case from 17 to 43 per litre. Such fluids clear on shaking out with ether. In other cases, however, the fluid does not contain nearly enough fat to explain the turbidity. By some lecithin is supposed to explain the cloudiness, but other fluids with much lecithin are clear and some milky fluids have very little (Christen). Others consider that globulin explains it, or casein or seromucoid. The most recent explanation is that it is a compound of globulin and lecithin, whether in combination or not is uncertain. Bernert, who examined one case with exceeding care, sums the matter up as follows: There are cases in which the milkiness is not due to fat alone, but to albumin of the globulin group from which large amounts of lecithin can be extracted by hot alcohol. The fat content is low, and resembles that of the so-called fatty degeneration of the epithelial cells. Quinke first showed that albumin also in fine granules could give a milky appearance.

The color of such fluids is white or yellowish-white, greenish or reddish, opalescent in thin layers. Some fluids become more milky on cooling. In some cases a perfectly clear fluid on the first tapping becomes progressively more milky on the subsequent tapplings. On standing the fluid will sometimes deposit a sediment and have a well-marked cream on the surface. Filtering or centrifugalizing does not clear it. The specific gravity varies from 1010 to 1014. In one case it was 1061, in another 1081, in which cases much pus must have been

present. Their reaction is alkaline, and, strange to say, there is no odor. In the cases that we have examined this has been a marked feature. They are very resistant against decomposition, and the fluids could remain in the laboratory for weeks without apparent change. The sediment is slight, consisting of epithelial cells, all degenerated with fatty globules, and globules which do not take the stains of fat.

In general there are two classes of cases,—those very milky, the “chylous,” and the “chyliform,” which are only very opalescent. In this case we used the terms only as descriptive without implying that chyle was or was not present.

The former occur when chyle is present, as in the traumatic cases; in others the fat may best be explained by the fatty globules freed from the fatty degenerated epithelium cells. But the majority of cases are hard to explain. Our best case was one of tuberculosis of the peritoneum. In Tabora's case of peritonitis carcinomatosa the fat was 1.2 per cent.; sugar, 0.864 per cent.

In our case of markedly chylous ascites the specific gravity was 1013.3; proteid, 5.114 gms. per litre; globulin, 73 per cent. of this. The fat-cholesterin-lecithin-fraction 1.469 per cent.

The opalescent fluids occur in a great variety of conditions, and are often found at autopsy; cachexias, anæmias, heart cases, etc.; Naunyn stated that the cause in many cases is amyloid degeneration of the blood-vessels of the serosa. The reason suggested for chylous fluids in cases of heart-failure is stasis in the thoracic duct. In other cases the stasis may be due to the pressure of tumors on the duct.

A chyliform ascitic fluid from a case of uræmia had a specific gravity of 1005.5, and solids 1.2988 per cent.

#### OVARIAN CYSTS

**COLLOID** is not one substance, the name being based on the physical properties of the contents of various cysts and organs. They are gelatinous, insoluble in water and acetic acid, soluble in alkali. From some may be split off a reducing body, but their composition varies much.

**PSEUDOMUCIN (METALBUMIN).**—This body occurs in many ovarian cyst contents which are very viscid and slimy. Alcohol gives a thready precipitate resembling wood-pulp, which can be wound around the rod. It is not precipitated by heat nor by acetic acid. The precipitate formed by alcohol is ground fine under alcohol, and then freed from alcohol by means of ether, and dissolved in water. It is then reprecipitated with alcohol. A light white powder is obtained which is soluble in water to an opalescent mucoid solution, which is not well precipitated by acetic acid. When boiled with HCl an abundant reducing body is split off, which reduces copper very easily.

**PARAMUCIN.**—This is a substance present in certain ovarian cysts, also in the ascitic fluid providing the ovarian cyst has already ruptured into the abdomen. It is firm, glistening, with the consistency of gelatin, soluble in dilute mineral acid, shrinks in acidulated alcohol, or in alcohol and ether, and can be reduced to a fine white powder. Its characteristics are, its insolubility in water, the fact that it swells in alkali dissolving in excess, that it is precipitated by acetic acid and is soluble in excess, and, especially, that it will reduce copper salts without preliminary boiling with acid.

**Serous Cysts** (dilatation of Graafian follicles) contain a perfectly clear serous fluid, watery, which foams easily, is of amber color, of a specific gravity from 1005 to 1022 (usually 1005 to 1014), with solids from 10 to 40 per litre and all the other constituents of serous fluids.

In two recent cases the specific gravity was 1022 in one case, 1016 in another; they contained a great deal of albumin. Heat alone caused but a faint cloud, but one drop of acid made the fluid perfectly solid. Both serum globulin and serum albumin were present; in these little or no euglobulin.

**Proliferating Cysts from Pfluger's Tubules.**—The contents of these are various. Some contain "colloid," and on boiling with acid give a

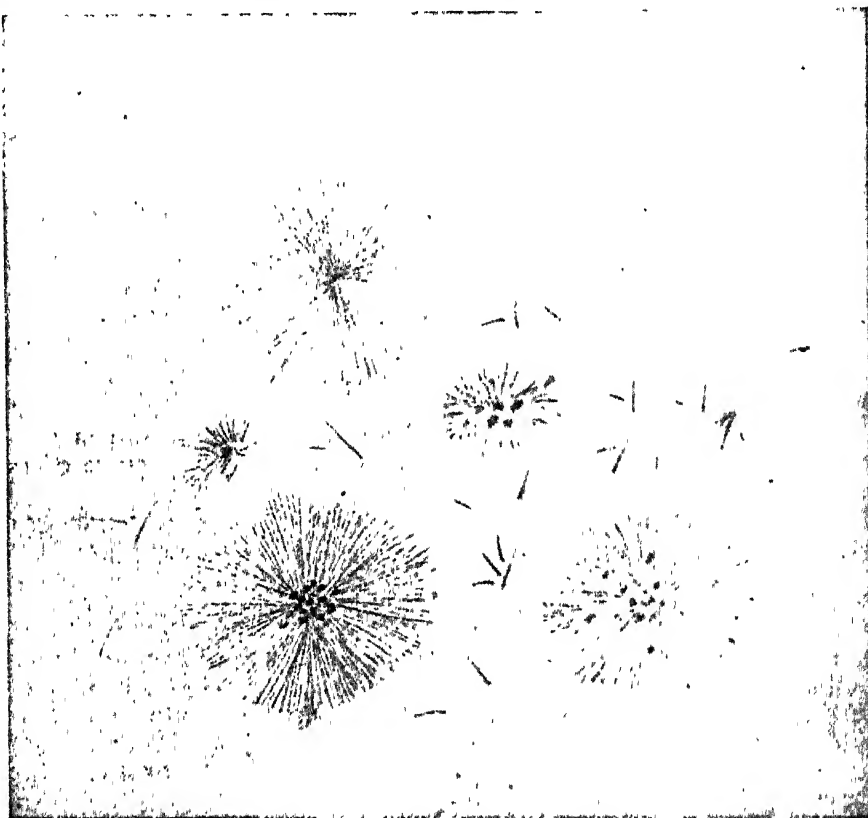


FIG. 124.—Fatty acid crystals from the contents of an ovarian cyst.  $\times 400$ .

reducing body. From the colloid ovarian cysts the fatty crystals (soluble on warming) may be watched to crystallize out singly and in rosettes (see Fig. 124).

Another group of cysts contain a viscid fluid, very stringy, which varies much in consistency according to the amount of serous fluid present. It is of a brownish or dark greenish-brown color.

The specific gravity of the contents in three recent cases was 1025 to 1030.2; the solids were 9.7 and 9.3 per cent.; the alcohol precipitate, 6.9 and 8.5 per cent.

Some, however, contain a thin watery fluid, of a bluish-white opalescent color which may, however, be yellowish, yellowish-brown, or greenish, according to the amount of blood present.

We give a few examples of the contents of such cysts which we have recently seen.

1. Fluid quite opalescent; specific gravity, 1004.3; solids, 2.837 per cent. Alcohol precipitate 1.98 per cent. resembles macerated filter paper, is not stringy, can be reduced to a fine white powder, difficultly soluble in water to an opalescent fluid. Watery extractives 0.524 (ash, 0.388) per cent. Alcohol-ether soluble extractives, 0.2056 (ash, 0.108) per cent.; fat, cholesterin, etc., 0.96 per cent.

2. Fluid reddish-yellow, considerable sediment of small epithelial and some large epithelial cells with coarse refractile granules; filters clear. Specific gravity, 1008.04; solids, 2.32 per cent. Alcohol precipitate similar to above.

3. Bluish opalescence; specific gravity, 1007.3.

Of some of the multilocular cysts the contents are thick, not especially viscid, but a suspension of glistening masses of cholesterin crystals and of a yellow-red or brown color, depending on the blood pigment.

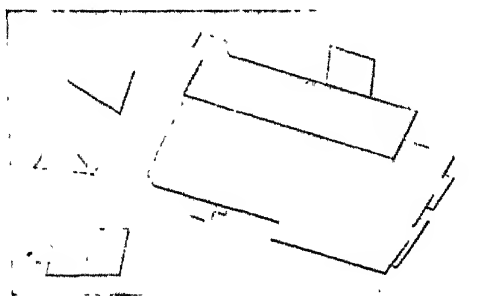


FIG. 125.—Cholesterin crystals.  $\times 400$ .

Of a recent case the figures were, specific gravity, 1025.9. The alcohol precipitate, 10.56 per cent.; contained no reducing body. Half saturation of the original filtered fluid with  $(\text{NH}_4)_2\text{SO}_4$  gave a precipitate 0.692 per cent. (globulin?); albumin? 0.604 per cent. Extractives, soluble in water, 1.46 (ash, 0.266) per cent.; alcohol-soluble extractives, 0.56 (ash, 0.44) per cent. Microscopically a great amount of detritus in the sediment, with very large cells (epithelial) full of glistening granules, cholesterin crystals, and fat needles.

In another similar case the specific gravity of the fluid was 1030.6.

The sediments contain much detritus, red blood-cells, leucocytes, large epithelial cells, single and in groups, filled with granules like fat, large masses of fatty granules, cholesterin crystals, and colloid granules which are large, circular, strongly refractive bodies.

In the case of a dermoid, the contents of which contained much paramucin, serum globulin and albumin could also be demonstrated. Water content of the jelly, 92.2 per cent. The alcohol precipitate in one case was 3.3 per cent. Water-soluble extractives, 0.4 (ash, 0.27) per cent. Alcohol-ether extractives, 0.25 (ash, 0.16) per cent.

**Tubo-ovarian Cysts.**—The contents of these are watery, thin, serous, and contain no pseudomucin.

**Parovarian Cysts.**—These contain a thin watery fluid of a very pale yellow or colorless or slightly opalescent appearance. Specific gravity, 1002 to 1009; solids from 10 to 20 per litre; no pseudomucin. Albumin may fail entirely or be only slight in amount. It consists, therefore, of water and extractives.

In a recent case, age twenty years, the cyst contained about 2 litres of very clear watery fluid with very slight opalescence; specific gravity, 1007.8; only the faintest precipitate with alcohol or ammonium sulphate; chlorides, 0.45 per cent. (as NaCl). Microscopically there were very few epithelial cells, round, granular, with a round nucleus.

**Intraligamentous Cysts.**—The contents of these are yellow, yellowish-green, or brownish. They contain little or no pseudomucin; specific gravity, 1032 to 1036; solids, 90 to 100 per litre, and the proteids of the blood plasma.

**Hydrocele.**—The contents are of a high color, clear or dark yellow, or greenish; specific gravity, 1014 to 1026; the solids on an average of 60 per litre. The fluid sometimes coagulates spontaneously. Leucocytes are always present, sometimes cholesterin crystals.

For illustration, in one case the specific gravity was 1010.7; solids, 6.329 per cent.; total albumin, 5.92 per cent., of which 45 per cent. was globulin; water-soluble extractives, 0.7504 (ash, 0.462) per cent.; alcohol-ether extractives, 0.452 (ash, 0.1726) per cent.; fat fraction, 0.1864 per cent.



FIG. 126.—Sodium biurate crystals from a tophus.  $\times 400$ .

**Spermatocele.**—The fluid of these cysts is colorless, watery, slightly milky; specific gravity, 1006 to 1010; average solids, 13 per litre; proteids slight, containing cell detritus, fat granules, and spermatozoa.

**Tophus.**—The tophi of gout, so important in diagnosis, can only be distinguished from small sebaceous cysts, small cartilaginous tumors, etc., by the microscopic examination of their contents. A little is mixed with water and found to be an amorphous paste, with many needles of sodium biurate (Fig. 126). Amorphous sebaceous matter with many fatty and cholesterin crystals is in sebaceous cysts.

The masses of **urea crystals**, the "urea frost," which appear on the skin of the face in rare cases of nephritis just before death (there have been but five cases in this clinic) may be tested by the method given on page 117. This is a most interesting phenomenon. The circulation in the skin is so poor when it occurs that it is very hard to believe that the immediate source of the urea is the blood.



# INDEX

## A

- Abscess, leucocytes in, 559  
   of kidney, blood in, 559  
   of liver, blood in, 648  
     sputum in, 81  
   of lung, leucocytes in, 559  
     sputum in, 80  
 Absolute amount of HCl in gastric juice, 361  
 Absorption power of stomach, 379  
 Accidental albuminuria, 234  
 Aceto-acetic acid, 199  
 Acetone, quantitative determination of, 199  
   in the urine, 194  
 Acholic stools, 404, 406  
 Achylia gastrica, 388  
 Acid, alloxypoteinic, 132  
   chrysophanic, 107  
   diacetic, in urine, 199  
   ferments, 168  
   glycuronic, 211  
   hippuric, 262  
   homogentisinic, 213  
   hydrochloric, 133, 355  
   lactic, 371, 393, 694  
   nitric, 147  
   nitrous, 148  
   oxalic, in urine, 261  
   oxybutyric, 202  
   oxypoteinic, 132  
   phosphoric, 137  
   silicic, 147  
   sulphocyanic, 147  
   sulphuric, 142  
   thiosulphuric, 147  
   uric, 121  
   uroleucinic, 213  
   values, 409  
 Acid-fast bacteria, 53  
 Acidity of gastric juice, 355  
   of urine, 107; determination of, 111  
   organic, in gastric juice, 375  
   total, of gastric juice, 355  
   values, 409  
 Acidophilic cells, 532  
 leucocytes, 532  
 Acidosis of diabetes, 209  
 Acids, bile, in stool, 406  
   in urine, 162  
   fatty crystals, in sputum, 31, 33  
   inorganic, of urine, 133  
 Acromegaly, blood in, 637  
 Actinomycosis of lung, sputum in, 42  
 Acute articular rheumatism, blood in, 631  
   bronchitis, 71  
 Acute diffuse nephritis, urine in, 317  
   diseases, blood in, 618  
   lobar pneumonia, sputum of, 58  
   luetie nephritis, urine in, 319  
 Acute miliary tuberculosis, blood in, 626  
   sputum in, 46  
   nephritis, urine in, 317  
   nephritis of cholera, urine in, 319  
   parenchymatous nephritis, 317  
 Acute pneumonic tuberculosis, 46  
   yellow atrophy of liver, blood in, 650  
 Addison's disease, blood in, 630, 649  
 Adenin, 127  
 Adenitis, tuberculosis, blood in, 618  
 Adolescence, albuminuria of, 234, 235, 238  
 Adrenals, tuberculosis of, blood in, 627, 649  
 Estivo-autumnal malaria, parasite of, 656, 664  
 Agar media, 293  
 Age, effect of, on count of reds, 514  
 Agglutination phenomena, 500  
 Agglutinins, 681  
 Agonal leucocytosis, 561  
 Air in sputum, 22  
 Albumin, calculi of, 288  
   determination of, in body fluids, 692  
   in stools, 397  
   quotient, 223  
   serum, 223  
   tests in urine, 214  
   to remove from urine, 223  
 Albuminous expectoration, 79  
 Albuminuria, 223  
   accidental, 234  
   adolescence, 234, 235, 238  
   after baths, 233  
   alimentary, 233, 245  
   anæmia due to, 558  
   cyclic, 234, 236  
   due to Bright's disease, 240  
   due to definite renal lesion, 240  
   due to palpation of kidney, 239  
   essential, 234  
   false, 230  
   febrile, 239, 316  
   functional, 231  
   hæmatogenous, 239  
   hereditary, 238  
 Albuminuria, hypostatic, 238  
   intermittent, 234, 238  
   luetie, 241, 319  
   minima, 238  
   nervous, 240  
   of apparently healthy, 234  
   of diabetes, 210  
   of labor, 234  
   of masturbators, 235  
   of new-born, 21  
   of puberty, 234  
   orthostatic, 234, 236  
   orthotic, 234  
   physiological, 234  
   post-infectious, 238  
   postural, 234, 236  
   structural, 231  
   traumatic, 239  
   true, 230  
   without definite renal lesion, 230  
 Albuminurie cicatricielle, 238  
   paracellaire, 238  
   phosphaturique, 238  
   prégoutteuse, 238  
   résiduale, 238  
 Albumosuria, 241, 243  
   alimentary, 245  
   enterogenous, 245  
   febrile, 245  
   hæmatogenous, 245  
   hepatogenous, 245  
   myelopathic, 241  
   pyogenic, 245  
 Alimentary albuminuria, 233, 245  
   albumosuria, 245  
   chloruria, 337  
   levulosuria, 189  
 Alkaline tide, 109  
 Alkalinity of blood, 573  
 Alkaptonuria, 212  
 Allantoin, 695  
 Alloxuric bases, 126  
 Alloxypoteinic acid, 132  
 Almén's test for glucose, 175  
 Aloin test, 411  
 Altitude, effect of, on count of reds, 516  
 Alveolar epithelial cells in sputum, 27  
 Alymphæmic lymphomatosis, 564  
 Amboceptors, 680  
 Ammonia in gastric contents, 375  
   in urine, 127  
 Ammonium biurate, 255  
   magnesium phosphate, 257  
 Amœba coli, 417  
   mitis, 419  
   pulmonalis, 43  
   vulgaris, 421  
 Amœbic dysentery, anæmia due to, 585

Amœbic dysentery, stools in, 442  
 Amount, absolute, of HCl in gastric juice, 361  
 of sputum, 18  
 of urine, 95  
 Amyloid kidney, urine in, 322  
 Anæmia, 575  
 chlorotic, 577  
 consumptive, 577  
 due to acute gastritis, 585  
 due to acute hemorrhage, 579  
 due to acute infections, 585  
 due to albuminuria, 586  
 due to amœbic dysentery, 585  
 due to bad air, 584  
 due to blood poisons, 582, 588  
 due to Bothriocephalus latus, 587  
 due to chronic gastritis, 585  
 due to chronic hemorrhage, 581  
 due to chronic infectious diseases, 585  
 due to coal-tar products, 589  
 due to constipation, 585  
 due to diarrhœa, 584  
 due to dilated stomach, 585  
 due to dysentery, 584  
 due to dyspepsia, 585  
 due to fever, 586  
 due to gastro-intestinal disorders, 584  
 due to intestinal parasites, 587  
 due to lack of sunlight, 584  
 due to lead, 588  
 due to poisons, 588  
 due to poor food, 583  
 due to pus formation, 586  
 due to Strongyloides intestinalis, 587  
 due to ulcerative colitis, 585  
 due to uncinariasis, 587  
 due to yellow fever, 588  
 hypoplastic, 577  
 of children, 634  
 of growth, 634  
 of the poor, 583  
 of the Tropics, 585  
 primary pernicious, 577, 589  
 pseudoleukæmia infantum, 635  
 secondary, 577  
 simple primary, 589  
 splenic, 589  
 Anæmic degeneration of cells, 509  
 Anaglycosuria, 340  
 Aniline gentian violet, 291  
 Ancestral corpuscles, 547  
 Angioneurotic hæmaturia, 247  
 Anguillula aceti in urine, 312  
 intestinalis, 431  
 stercoralis, 431  
 Animal gum, 194

Animal gum, parasites in sputum, 43  
 in stools, 417  
 in urine, 310  
 Ankylostomum duodenale, 427  
 Anopheles mosquito, 661  
 Anthracosis, 21, 87  
 Antibodies, 680  
 Antigens, 680  
 Antitoxins, 681  
 Anuria, 98  
 Appearance, general, of urine, 94  
 Appendicitis, blood in, 633  
 Arabinose in urine, 191  
 Arsenic in urine, 150  
 Arteriosclerosis of kidney, urine in, 321  
 Arthritis deformans, blood in, 633  
 Arthus and Huber method (trypsin), 399  
 Ascaris lumbricoides, 425  
 texana, 425  
 Ascitic fluid, cyto-diagnosis of, 701  
 Aseptic condition of stomach, 352  
 Asiatic cholera, stools in, 438  
 Aspergillus flavus, 38  
 fumigatus in sputum, 38  
 glaucus, 38  
 niger, 39  
 subfuscus, 39  
 Assimilation limit for sugars, 169  
 Asthma, blood in, 633  
 eosinophilia in, 565  
 sputum of, 67  
 Atony of stomach, 380  
 Atrophy, acute yellow, of liver, blood in, 650  
 of mucosa, 388  
 renal, 324  
 Auer's bodies, 615  
 Avirulent diphtheria bacillus, 88  
 Azotorrhœa, 412

## B

Bacillus aërogenes capsulatus, 296  
 alkaligenes, 296  
 bifidus, 436  
 Bordet's, 66  
 buccalis maximus, 36  
 Bacillus coli communis, 234  
 diphtheriæ, 87  
 fusiformis, 92  
 Kauffman's, in gastric juice, 395  
 of influenza, 65, 701  
 lactis aërogenes, 295  
 lepræ, 54  
 paratyphosus, 295  
 pseudodiphtheriæ, 88  
 pyocyaneus, 296  
 tetani, 297  
 tuberculosis, 49  
 typhosus, 295  
 ulceris canceri, 308  
 Bacteria, acid fast, 51  
 media for, 293  
 in sputum, 34  
 stains for, 290  
 in stomach, 378  
 in stools, 402  
 in urine, 298

Bacteriology, of blood, 498  
 Bacteriolysins, 681  
 Bacteriorrhœa, 306  
 Bacteriuria, 302  
 Balantidium coli, 424  
 Bases, alloxuric, 126  
 inorganic, in urine, 133  
 nucleic, 126  
 of gastric juice, 375  
 purin, 126  
 xanthin, 126  
 Basophile granules in red cells, 511  
 of leucocytes, 534  
 Basophilia, 508  
 Baths, albuminuria following, 233  
 effect of, on red count, 517  
 Bence-Jones's body, 241  
 Benedict's method for determination of glucose, 182  
 Benzidin test for blood, 412  
 Blal's test for pentose, 192  
 Bile acids in stools, 390  
 in urine, 162  
 in vomitus, 406  
 pigments in urine, 156  
 stained sputum, 20  
 tests for in urine, 158  
 to remove from urine, 161  
 Bilharzia eggs in urine, 311  
 Bilifuscin, 156, 157  
 Biliprasin, 156, 157  
 Bilirubin, in blood, 495  
 in stools, 405  
 in urine, 156, 161  
 in urine sediment, 262  
 Biliverdin, 156, 157  
 Bismuth crystals in stools, 414  
 Biturate of ammonia, 255  
 Biturates, 123  
 Biuret test, 121  
 Black's method, qualitative determination of oxybutyric acid, 202  
 quantitative determination of oxybutyric acid, 203  
 Black urines, 106  
 Blackwater fever, 248  
 Bladder stones, 286  
 tuberculosis of, 300  
 Blastomycetes, 40  
 in blood, 588  
 Blastomycosis, 40  
 Blood, 447  
 agar, 294  
 alkalinity of, 542  
 bacteriology of, 498  
 bilirubin in, 495  
 chemical tests for, 249  
 coagulation of, 487  
 examination, value of, 650  
 freezing point of, 332  
 fresh, study of, 329, 331  
 in abscess of liver, 648  
 in acromegaly, 637  
 in acute articular rheumatism, 633  
 in acute diseases, 618  
 in acute yellow atrophy, 650

Blood, in Addison's disease, 627, 649  
in appendicitis, 633  
in arthritis deformans, 633  
in bronchial asthma, 633  
in bronchopneumonia, 631, 632  
in cancer, 638  
  of breast, 641  
  of intestines, 643  
  of œsophagus, 642  
  of rectum, 643  
  of stomach, 641  
  of testicle, 643  
in catarrhal jaundice, 648  
in cholangitis, 648  
in cholecystitis, 648  
in chorea, 636  
in chronic diseases, 636  
in chronic septicæmia, 621  
in cirrhosis of liver, 648  
in depressive insanity, 637  
in diabetes mellitus, 637  
in diphtheria, 623  
in diseases of liver, 648  
in gall-stone colic, 648  
in general paresis, 636  
in German measles, 622  
in heart disease, 649  
in leprosy, 649  
in lues, 643  
in lues, congenital, 635  
in malaria, 618  
in malignant disease, 638  
in measles, 622  
in myxœdema, 650  
in nephritis, 648  
in nervous diseases, 636  
in pneumonia, 629  
in renal diseases, 646  
  acute nephritis, 646  
  bilateral cystic kidney, 648  
  chronic nephritis, 647  
in rickets, 636  
in sarcoma, 643  
in scarlet fever, 623  
in scurvy, 650  
in septicæmia, 621  
in smallpox, 623  
in summer diarrhœas, 636  
in toxic jaundice, 648  
in tuberculosis, 624  
  acute miliary, 626  
  of adrenals, 627  
  of bones and joints, 627  
  of intestines, 627  
  of lungs, 625  
  of meninges, 627  
  of peritoneum, 627  
  renal, 627  
in typhoid fever, 627  
in typhus fever, 622  
reaction of, 572

Blood, red cells of, 450, 505  
  tests for Almén's, 250  
    guaiac, 250  
    hæmin, 250  
    Heller's, 249  
    Schönbein's, 250  
    spectroscopic, 251  
    Teichmann's, 250  
  urea in, 575  
  urobilin in, 495  
  viscosity of, 492  
Blood-casts, 276  
Blood-cells in gastric contents of cancer or stomach, 396  
  in gastric juice, 378  
  in sputum, 29  
  in stools, 410  
  in urine, 246  
  in vomitus, 351  
Blood-clots in stomach, 396  
Blood-crises, 543, 594, 596  
Blood-cultures, 490  
Blood-platelets, 568  
Blood-serum, Löffler's, 87  
Blood-smears, 474  
Blood-staining, 478  
Blue, indigo, 152  
  urines, 100  
Boas bacillus (Oppler), 395  
  evening meal, 381  
  meal (free lactic acid), 373  
  method (lactic acid), 374  
Bodies, foreign, in sputum, 26  
Bogg's methods for preserving and mounting worms, 445  
Bone marrow, 541  
  diseases of, eosinophilia in, 561  
Bones, tuberculosis of, blood in, 627  
Borlet-Gengou phenomenon, 679  
Bothriocephalus latus, 434  
  cause of anæmia, 587  
Bouillon, 294  
Bound HCl, 359  
Braduria, 211  
Breakfast, test, 353  
Breast, cancer of, blood in, 641  
Bremer's blood test, 638  
Bright's disease, albuminuria of, 240, 315  
  blood in, 646  
Brodie-Russel coagulometer, 489  
Bronchial asthma, blood in, 565, 643  
  catarrh, desquamative, 71  
  colic, 26  
Bronchiectasis, 77  
  hemorrhage in, 78  
Bronchioliths, 25  
Bronchitis, acute, 71  
  capillary, 73  
  chronic, 73  
  croupous, 76  
  eosinophiles in, 27  
  fetid, 75  
  fibrinous, 76  
  plastic, 76  
Bronchoblenorrhœa, 75  
Bronchopneumonia, blood in, 631

Bronchopneumonia, sputum of, 64  
  tuberculous, sputum of, 47  
Bronchorrhœa, 75  
  humidum, 75  
  serosa, 75  
Buccalis maximus, bacillus, 36  
Buerger's capsule stain, 63  
Butyric oxy-acid in urine, 202

C

Cachexia, effect of, on red count, 517  
Calcium carbonate sediments, 252  
  of urine, 144  
  oxalate crystals in sputum, 34  
  phosphate sediments, 258  
  stones, 288  
  sulphate sediments, 261  
  urate sediments, 256  
Calculosa, pseudophthisis, 25  
Calculus, renal, 286  
  leucocytes in, 559  
  ureteral, 327  
Cancer fragments in gastric juice, 395  
  in urine, 284  
  of breast, blood in, 641  
  of intestines, blood in, 652  
  of kidney, urine in, 325  
  of lung, sputum of, 86  
  of œsophagus, blood in, 642  
  of rectum, blood in, 643  
  stools in, 439  
  of stomach, blood in, 641  
  blood in gastric contents, 396  
  flagellates in, 397  
  gastric juice in, 391  
  of testicle, blood in, 643  
Capillary bronchitis, 73  
Capsule stains, 62  
Carbohydrates in stools, 412  
  in urine, fermentable, 169  
  unfermentable, 204, 212  
Carbolfuchsin, 92, 291  
Carbol-thionin stain, 482  
Carbonates in sediments, 257  
  in urine, 147  
Carbon-monoxide poisoning, effect of, on red count, 518  
Cardiac disease, blood in, 649  
Carnin, 126  
Casts, 275  
  blood, 276  
  chemistry of, 280  
  colloid, 276  
  combined, 278

- Casts, diagnostic importance of,** 280  
 epithelial, 274  
 fatty, 275  
 fibrinous, in sputum, 24  
 glassy, 276  
 granular, 274  
 hyaline, 276  
 of moulds in sputum, 24  
 origin of, 279  
 prostatic, 314  
 pus, 276  
 size of, 278  
 staining, 282  
 testicular, 283  
 urate, 278  
 waxy, 275  
**Catarrh, desquamative of,**  
 bowel, 415  
 desquamatory, bronchial, 71  
 dry, 74  
 sec, 74  
**Catarrhal jaundice, blood in,** 648  
 pneumonia, desquamatory, 28  
**Catheterization, technic,** 289  
**Cavity formation in tuberculosis,** 57  
**Cells, epithelial, in prostatic fluid,** 313  
 in urine sediment, 271  
**Centrifugalization of urine,** 290  
**Centrifuge, quantitative determination of albumin,** 222  
**Cercomonads in sputum,** 43  
 in urine, 310  
**Cercomonas coli,** 422  
 hominus, 424  
 intestinalis, 422  
**Cerebrospinal fluid,** 696  
 meningitis, leucocytes in, 556  
**Cestodes,** 432  
**Chalcosis,** 21, 87  
**Chancres, organisms of,** 308  
**Character of sputum,** 19  
**Characteristics of urine in general,** 94  
**Charcot-Leyden crystals in blood,** 609  
 in sputum, 34, 70  
 in stools, 414  
**Chemistry of casts,** 280  
**Child eosinophilia of,** 564  
**Children, anæmia of,** 634  
 sputum, of 18  
**Chloride, excretion in urine,** 133  
 excretion tests, 337  
**Chloroma,** 21  
**Chlorosis,** 601  
**Chlorotic anæmia,** 577  
**Chloruria, alimentary,** 337  
**Cholangitis, blood in,** 648  
**Cholecyanin,** 156, 157  
 test for bile, Stokvis, 160  
**Cholecystitis, blood in,** 638  
**Cholera, acute nephritis of,** 319  
**Cholera spirillum,** 438  
 leucocytes, 557  
**Cholera infantum, blood in,** 636  
**Cholesterin in sputum,** 33  
 in stools, 414  
 in urine sediment, 263  
**Cholesterinuria,** 263  
**Choletelin,** 156, 158  
**Cholin,** 698  
**Chorea, blood in,** 636  
**Chromogens, color due to,** 21, 35  
**Chronic bronchitis,** 73  
 diseases, blood in, 636  
 gastritis, 386  
 interstitial pneumonia, sputum of, 64  
 nephritis, 319  
 passive congestion of lung, sputum of, 83  
 urine in, 316  
 ulcerative tuberculosis, sputum in, 47  
**Chrysophanic acid in urine,** 107  
**Chyliform fluids,** 707  
**Chylous fluids,** 706  
**Chyluria,** 269  
**Cicatricelle albuminurie,** 238  
**Cimænomonas hominus,** 415  
**Cirrhosis of liver, blood in,** 648  
**Clay-colored stools,** 404, 406  
**Cleaning glass,** 448  
**Clots, blood, in gastric juice,** 396  
**Cloudy swelling of kidney, urine in,** 316  
**Coagula of albumin in stools,** 412  
 of fibrin in sputum, 24  
**Coagulation of blood,** 487  
**Coal pigment in sputum,** 21, 28  
**Coal-tar products, anæmia, due to,** 589  
**Coccidiosis,** 41  
**Coefficient of Hauser,** 101  
**Colic, bronchial,** 26  
**Colica mucosa,** 410  
**Colitis anæmia due to,** 585  
**Collection of urine,** 94  
**Colloid casts,** 276  
**Colon bacillus,** 294  
**Color index of blood,** 530  
 of sputum, 19  
 of stools, 404  
 of urine, 101  
 due to medicines, 106  
**Coma, diabetic,** 209  
 leucocytes in, 559  
**Combined casts,** 278  
**Complements,** 80  
**Complement, fixation of,** 682  
**Concretions,** 286  
 acid calcium phosphate, 287  
 albumin, 288  
 ammonium urate, 286  
 calcium carbonate, 287  
 oxalate, 287  
 cystin, 287  
 fatty, 288  
 in bowel, 416  
 indigo, 288  
 phosphate, 287  
 renal, 286  
 table for detection of, 288  
 triple phosphate, 287  
**Concretions, uric acid,** 286  
 vesical, 286  
 xanthin, 287  
**Conductivity, electrical,** 335  
**Congenital cystic kidney,** 325  
 heart disease, effect of, on red count, 518  
**Congestion, chronic, passive, of lung,** 85  
 urine in, 316  
**Consistency of sputum,** 19  
 of stools, 402  
**Constipation,** 403  
 anæmia, due to, 585  
 latent, 399  
**Constituents of normal stools,** 402  
**Consumptive anæmia,** 361  
 contents of stomach, examination of, 350  
**Continuous secretion,** 383  
**Corpora amylacea in prostatic fluid,** 313, 314  
**Cough, whooping, sputum of,** 67  
**Counting red cells,** 459  
**Creatinin,** 130  
**Crenated red cells,** 454  
**Crescentic bodies in red cells,** 455  
**Crises, blood,** 543, 594, 596  
**Croupous bronchitis, sputum in,** 76  
 pneumonia, blood in, 629  
 sputum, of 58  
**Cryoscopy of urine,** 329  
 of blood, 332  
**Crystals, Charcot-Leyden, in blood,** 609  
 in sputum, 34  
 in stools, 609  
 fatty acid, in sputum, 31, 33  
 in gastric juice, 377  
 in sputum, 33  
 in stools, 413  
 melting point, determination of, 178  
 spermin, in prostatic fluid, 314  
**Cultures of blood,** 499  
**Culture media,** 293  
**Cultures of throat,** 87  
 urine, 289  
**Curds in stools,** 412  
**Curschmann's spirals,** 23, 68  
**Cyanin blue stain,** 291  
**Cyanosis, effect of, on red count,** 518  
**Cyclic albuminuria,** 234, 236  
**Cylindrical epithelial cells in sputum,** 27  
 epithellum in stools, 413  
**Cylindroids,** 277  
**Cylindruria,** 281  
**Cystic kidney, congenital, urine in,** 325  
**Cystin in urine,** 266  
 stones, 287  
**Cystinuria,** 266  
**Cystitis, cultures in,** 300  
**Cysts, echinococcus, in sputum,** 26  
**Cytodiagnosis of ascitic fluid,** 703

Cytodiagnosis of cerebro-spinal fluid, 700  
of pleural fluid, 703

D

Dahlia stain, 482  
Dare's alkalimeter, 574  
  hæmoglobinometer, 526  
Day and night urine, 96  
Deen's test, 379  
Deficit of  $\text{HCl}$ , 359  
  saturation, 359  
Degeneration, amyloid, urine in, 322  
  anæmic, of red corpuscles, 509  
Degenerations of red cells, 454, 455  
Delayed urea excretion, 336  
Dementia præcox, leucocytes in, 559  
Desmold test, 352  
Desquamative nephritis, urine in, 317  
Desquamatory bronchial catarrh, 71  
  catarrhal pneumonia, 28  
Deutero-albumosuria, 243  
Diabetes insipidus, 210  
  mellitus, 203  
  blood in, 637  
Diabetic coma, 209  
  leucocytes in, 559  
Diabetic acid, 199  
Diagnosis, functional renal, 328  
  surgical, value, 347  
Diamines in urine, 267  
Diarrhœa, 403  
  anæmia due to, 584  
  of children, blood in, 636  
  pancreatica, 407  
Diastase in stools, 413  
  in urine, 168  
Diazo-test, urine, 163  
Dicalcium phosphate sediment, 258  
Differential counting, 538  
Differentiated inner body of erythrocytes, 506  
Diffuse nephritis, acute, urine in, 317  
Digestion, gastric, extent of, 371  
  products of, 372  
  leucocytosis, 553  
  starch, 371  
Digestive power of pancreatic juice, 400  
Dilated stomach, anæmia due to, 371  
  contents of, 382  
Dilution test of urine, 337  
Dimethylamidoazobenzol, 356  
Dimethylamidobenzaldehyde test, 167  
Diphtheria, 87  
  bacillus, 87  
  blood in, 623  
Diplococcus lanceolatus in sputum, 62  
Diplococcus pneumoniae, 62  
Dipylidium caninum, 434  
Diseases of kidneys, urine in, 315  
Distoma hæmatobium kidney, 435

Distoma hepaticum, 432  
  lanceolatum, 432  
Distribution of nitrogen in urine, 120  
Ditrich's plugs, 23  
Dried residue, determination of, 692  
Drigalski and Conradi's medium, 437  
Dropsical cells, 507  
Drugs, effect of, on red count, 517  
Dry catarrh, 74  
Ducrey's bacillus, 308  
Duodenal ulcer, 390  
Dysentery, anæmia due to, 584  
  bacilli of, 442  
  stools in, 439  
Dyspepsia, anæmia due to, 585  
  nervous, 385

E

Easily split sulphates, 147, 151  
Echinococcus disease of kidney, 310, 327  
  of lung, sputum in, 26, 43  
Eclampsia, urine, 324  
Ectasis of stomach, 380  
  hypertonic, 380  
Eel, vinegar, in urine, 312  
Effusion, pleural, leucocytes in, 538  
Egg-yellow reaction, 167  
Egyptian hæmaturia, 311  
Ehrlich's classification of leucocytes, 535  
  egg-yellow reaction, 167  
  stains, 479  
Einhorn fermentation tubes, 191  
Elastic tissue in sputum, 29, 48  
  pseudo-, in sputum, 31  
Electrical conductivity, 335  
Emphysema, chronic bronchitis of, 70, 74  
Empyema, leucocytosis in, 538  
  perforating lung, 82  
Emulsions of leucocytes, 676  
Endocarditis, leucocytes in, 557  
Entamœba coli, 421  
  dysenteriae, 421  
  histolytica, 421  
Enteritis membranacea, 410  
Enterogenous albumosuria, 245  
Enteroliths in stools, 416  
Einthelmintha, 425  
Eosinophile granules, 532  
  leucocytes, 536  
Eosinophilia, 564  
  after tuberculin reaction, 566  
  bronchitis, 27  
  due to parasites, 565  
  in asthma, 565  
  in diseases of bone marrow, 564  
  of genital organs, 566  
  of hæmatopoietic organs, 564

Eosinophilia of lymph glands, 565  
  of spleen, 564  
  of sympathetic nervous system, 567  
  in malignant disease, 566  
  in skin disease, 565  
  medicinal, 566  
  physiological, 564  
  post-febrile, 566  
Eosinophilic bronchitis, 27  
Epieritric polyuria, 97  
Epiguanin, 126  
Episarken, 126  
Epistaxis, Gull's renal, 246  
Epithelial casts, 274  
  cells in gastric contents, 378  
  in prostatic fluid, 313  
  in sputum, 27  
  in stools, 413  
  of urine sediments, 271  
  filtration, 338  
Erosions, hemorrhagic, of gastric mucosa, 390  
Erysipelas, leucocytes in, 557  
Erythroblasts, 548, 549  
Erythrocytosis, 518  
Erythrodestrin, 193  
Esbach tubes, 221  
Essential albuminuria, 234  
  renal hæmaturia, 246  
Ethereal sulphates, 142, 145  
Euglobulin, 225, 702  
Eustrongylus gigas in kidney, 311  
Ewald-Boas test breakfast, 353  
Exercise, leucocytosis due to, 561  
Extent of gastric digestion, 371  
Extraneous structures in sputum, 26  
Exudates, 702

F

Fæces, 399  
False albuminuria, 230  
Famine fever, 671  
Fasciola hepatica, 432  
Fasting stomach, contents of, 353  
Fat in blood, 638  
  in body fluids, 693  
  in stools, estimation of, 409  
Fat-crystals of stools, 414  
Fate of nucleus of erythrocytes, 545  
Fat-splitting ferment in gastric juice, 370  
Fatty acid crystals in sputum, 31, 33  
  acids in stools, estimation of, 409  
  casts, 275  
  cells in sputum, 28  
  concretions in urine, 288  
  granules in leucocytes, 534  
  kidneys, urine of, 316  
  stools, 406  
Febrile albuminuria, 239  
  albumosuria, 245  
  diseases, leucocytes in, 555

- Fecal vomitus, 352  
 Fehling's test, determination of sugar, 184  
 Fermentation in gastric juice, 376  
 Ferment, fat-splitting, in gastric juice, 368  
     of urine, acid, 111  
 Ferments in stools, 413  
     in urine, 168  
 Ferrocyanide test, albuminuria, 218  
 Ferrometer, Jolles's, 529  
 Fetid bronchitis, 75  
 Fever, blood in, 586  
 Fibres, muscle, in gastric juice, 377  
     in stools, 412  
 Fibrin casts in sputum, 24  
     coagula in sputum, 24  
     diagnosis, 491  
     network in fresh blood, 459  
     structures in sputum, 24  
 Fibrinogen, 224  
     in body fluids, 692  
 Fibrinoglobulin, 224  
 Fibrinous bronchitis, 76  
 Fibrinuria, 230  
 Fibroid form of pulmonary tuberculosis, 46  
 Filaria bancrofti, 669  
     demarquai, 671  
     diurna, 670  
     gigas, 671  
     loa, 671  
     megalhæsi, 671  
     nocturna, 670  
     ozzardi, 671  
     perstans, 670  
 Filariasis, 669  
 Filtration, epithelial, kidney, 338  
 Fischer's test meal, 354  
 Fistula, jejunal, 402  
 Fixation of complement, 682  
 Fixing methods for smears, 476  
 Flagellata in cancer of stomach, 397  
     in sputum, 43  
     in stools, 421  
     in urine, 310  
 Flagellum stains, 291  
 Fleischl hæmoglobinometer, 523  
 Fluid, cerebrospinal, 696  
     gastric, 350  
 Fluid, pancreatic, 399  
     prostatic, 312  
     body, analysis of, 692  
 Fluke, lung, 44  
 Fœtal blood, 551  
 Folin's, acetone method, quantitative determination, 199  
     ammonia method, 129  
     standard diet, 400  
     urea method, 119  
 Follicular tonsillitis, blood in, 556  
 Foreign bodies in sputum, 26  
 Form of stools, 403  
 Fragments of cancer in gastric juice, 395  
     of mucosa in gastric juice, 377  
     of tissue in sputum, 22  
     in sputum of abscess of lung, 81  
 Fragments of tissue in sputum of gangrene of lung, 81  
     in stools, 417  
     in urine, 284  
     in vomitus, 353  
 Free HCl, determination of, 358  
 Freezing point of urine, 329  
 Frequency of defecation, 403  
 Fresh blood, study of, 450  
 Freund's method of determining the acidity of urine, 141  
 Fuchsin, 291  
 Fuchsinophilia, 508  
 Fuchsinophilic cells, 543  
 Functional albuminuria, 231  
     renal diagnosis, 328  
     surgical value of, 347  
 Furbringer's hooks, 300-306  
 Furfurol test, 122
- ### G
- Gabbett's methylene blue, 52  
 Gaffky's table, 56  
 Gall sand, 415  
 Gall-stone colic, blood in, 648  
 Gall-stones, 414  
     pseudo-, 415  
 Gamete, 652  
 Gametozont, 652  
 Gangrene of lung, leucocytes in, 559  
     sputum of, 79  
 Gas bacillus, 295  
 Gastric contents, 350  
     acidity, 353  
     abnormalities, 382  
     bacteria in, 378  
     blood in, 378  
     blood-clots in, 396  
     cancer ferments in, 395  
     crystals in, 377  
     epithelial cells in, 378  
     fragments of mucosa, 377  
     infusoria in, 377  
     moulds, yeasts, sarcina in, 378  
     mucus in, 387  
     muscle fibres in, 377  
     pus in, 377  
     diagnosis without tube, 364  
     digestion, extent of, 371  
     products of, 371  
     juice, acidity of, 355  
     ammonia in, 375  
     bases of, 375  
     fermentation of, 376  
     ferments, 156  
     physiology of, 362  
     motility, 380  
     mucosa, atrophy of, 388  
     ulcer, 389  
 Gastritis acida, 387  
     acuta, 386  
     chronica, 386  
 Gastritis phlegmonosa, 386  
     purulenta, 386  
     acute and chronic, anæmia due to, 585  
 Gastro-intestinal disorders, anæmia due to, 584  
 Gastroenterorrhœa, 383  
 Gastroxynsis, 383  
 Gelatine, 294  
 General paresis, blood in, 636, 559  
     spinal fluid in, 701  
 Genitalia, eosinophilia in diseases of, 566  
 Genitalia, infection of, 303, 308  
 Gentian violet, 291  
 Gerhard's test, diacetic acid, 201  
 German measles, blood in, 622  
 Giant cells of bone marrow, 550  
 Giemsa's stain, 309  
 Gigantocytes, 507  
 Glanders of lung, 67  
 Glassy casts, 276  
 Globular richness, blood, 597  
 Globulin in body fluids, 692  
     serum, in urine, 224  
 Glomerular insufficiency, 337  
 Glucose, 170  
 Glutoid capsules, 400  
 Gluzinski's test, 397  
 Glycerine agar, 294  
 Glycogen in urine, 193  
 Glycoproteids, 692  
 Glycosuria, 170  
 Glycuronic acid, 211  
 Gonococcus, 304  
 Gonorrhœal cystitis, 301  
 Gout, leucocytes in, 559  
 Gouty albuminuria, 238  
 Gowers's hæmoglobinometer, 524  
 Gram's stain, 88  
 Gram-negative organisms, 88  
 Gram-positive organisms, 88  
 Granular casts, 274  
     cells in prostatic fluid, 313  
     masses of Schultze, 568  
 Granules, acidophile, 532  
     basophile, 534  
     of Grawitz, 511  
     eosinophile, 532  
     fatty, 570  
     Grawitz, 511  
     hæmokonien, 458  
     in red cells in malaria, 511  
     Mastzell, 532  
     neutrophile, 534  
     of red cells, 456, 510, 511  
     oxyphilic, 532  
     perinuclear, 534  
     pigment, in sputum, 28  
     sago, in sputum, 28  
 Grawitz granules, 511  
 Grawitz's unripe cell, 548  
 Green sputum, 20, 60  
     vomitus, 351  
 Grinder's rot, sputum of, 21  
 Grosse's method pepsin determination, 368

Growth, anæmia of, 634  
Gruber-Widal test, 500  
Guaiac test, 250  
Guanin, 126  
Gull's renal epistaxis, 246  
Gum, animal, in urine, 194  
Gunning's test, acetone, 196  
Gunzburg's reagent, 356

## H

Hæmamœba leukæmia magna, 616  
parva, 617  
malaria, 664  
virax, 652  
Hæman test, blood in urine, 250  
Hæmatochyluria, 670  
Hæmatocrit, 471  
Hæmatogenous albuminuria, 239  
albumosuria, 245  
jaundice, 156  
Hæmatoidin in sputum, 20, 33  
in urine sediment, 262  
Hæmatopoietic organs, 576  
eosinophilia in diseases of, 564  
Hæmatoporphyrin, 252  
Hæmatozoon falciparum, 657  
Hæmaturia, 246  
angioneurotic, 247  
Egyptian, 306  
essential, 246  
renal, 246  
Hæmolytic system, 682  
Hæmoglobin, 520  
estimation of, 520  
in sputum, 20  
in urine sediment, 263  
Hæmoglobinæmia, 493  
Hæmoglobinæmic degenerations, 455  
Hæmoglobinometers, 520  
Hæmoglobinuria, 247  
paroxysmal, 248  
Hæmoglobinuric nephritis, 318  
Hæmokonien granules, 458  
Hæmophilia, renal, 247  
Hæmophysical phthisis, 58  
Hæmoptysis, 84  
parasitic, 44  
Hæmosiderin crystals, 458  
Hæmmersten's bile test, 162  
Hammerschlag's method of determining pepsin, 367  
of determining specific gravity of blood, 483  
Hard chance, organism of, 308  
Haser's coefficient, 101  
Hayem's fluid, 460  
hæmocytometers, 470  
Hay's bile acid test, 163  
Healthy albuminuria of apparently, 234  
Heart disease, blood in, 551  
effect of, on count of reds, 518  
Heat test, albumin, 215  
glucose, 181  
Heating smears, 476  
Hehner-Maiv method, organic acids, 375

Hehner's value, 306  
Heller's test, albumin, 216  
quantitative, 221  
blood, 249  
Hemolytic serum, 684  
Hemorrhage, anæmia due to, 579  
in bronchiectasis, 78  
occult, in stools, 378  
pulmonary, in tuberculosis, 58  
Hemorrhagic erosions of gastric mucosa, 390  
infarct of lung, 85  
nephritis, 246  
pneumonia, 60  
sputum, 19, 84  
Hepatogenous albumosuria, 245  
jaundice, 155  
Hereditary albuminuria, 238  
Herzfehlzellen, 29, 86  
Hetero-albumose, 262  
Hetero-albumosuria, 241  
Heterochylia, 383  
Heteroxanthin, 126  
Hippuric acid, 262  
test, 338  
His's capsule stain, 63  
Hodgkin's disease, 617  
Homogentisinic acid, 213  
Huefner's method of determining urea, 119  
Hühnerfeld's solution, 379  
Hunger diabetes, 170  
Huppert's bile test, 160  
Hyaline casts, 276  
Hydræmia, 576  
Hydrocele fluid, 710  
Hydrochinone, 105, 155  
Hydrochloric acid in gastric juice, 356  
absolute amount, 361  
bound, 359  
deficit, 359  
quantitative determination of, 358  
Hydrogen sulphide in urine, 147  
Hydronephrosis, 327  
Hygrometry, 485  
Hymenolepis nana, 323  
Hyperaciditas hydrochlorica, 382  
Hyperacidity, 382  
Hyperchlorhydria, 382  
Hyperkinesis, 383  
Hypermotility, 380  
Hypersecretion, 383  
Hypertonic ectasis, 380  
Hypocythæmia, 576  
Hypoplastic anæmia, 577  
Hypostatic albuminuria, 238  
Hypoxanthin, 127

## I

Immature nucleated reds, 542  
Immune bodies, 672  
Importance of casts, 280  
Inanition, anæmia of, 583  
Index, color, 530  
volume, 531  
Indifferent lymphoid cells, 548  
Indigo calculi, 288  
carmine test, 340  
sediment, 263

Indigo-blue in urine, 152  
-red in urine, 154  
Indoxyl sulphate in urine, 150  
Indurative nephritis, urine in, 320  
pneumonia, sputum of, 64  
Infarction of kidney, urine in, 326  
Infectious disease, cause of anæmia, 585  
Infectious nephritis, urine in, 299  
Inflammatory leucocytosis, 555  
Influenza, bacillus of, 65, 701  
leucocytes in, 558  
sputum of, 64  
Influenzal meningitis, 701  
Infusoria in cancer of stomach, 397  
in intestine, 417  
in sputum, 43  
Inorganic acids in urine, 133  
bases in urine, 133  
Inoscopy, 705  
Inosite, 694  
in urine of diabetes insipidus, 211  
Insanity, blood in, 637  
Insipidus, diabetes, 210  
Insufficiency, glomerular and tubular, 337  
motor, of stomach, 380  
Intermediary bodies, 681  
Intermediate forms of nucleated reds, 543, 596  
Intermittent albuminuria, 234, 238  
Interstitial gastritis, purulent, 386  
nephritis, urine in, 321  
pneumonia, sputum of, 64  
Intestinal cancer, blood in, 643  
concretions, 416  
obstruction, leucocytes in, 557  
parasites, 417  
cause of anæmia, 587  
sand, 416  
test meals, 400  
worms, 425  
Intestine, contents of, 405  
motility of, 405  
tuberculosis of, blood in, 627  
Intraligamentous ovarian cysts, fluid of, 710  
Iodide of potassium test, 340  
Iodine reaction in blood, 567  
Iodophilia, 567  
Iron in urine, 149  
Irritation forms of leucocytes, 537  
Isomaltose, 194

## J

Jaffé's test for creatinin, 131  
for indoxyl sulphate, 152  
Jaundice, catarrhal, blood in, 648



Jaundice, hæmatogenous, 156  
 hepatogenous, 155  
 toxæmic, 156  
 toxic, blood in, 648  
 Jejunal fistula, 402  
 Jenner's stain, 482  
 Joints, tuberculosis of, blood in, 627  
 Jolles' ferrometer, 529  
 test albuminuria, 210  
 Juice, gastric, acidity of, 355  
 Justus's test, 645

## K

Kahler's disease, 241  
 Kaufmann's bacillus, 395  
 Kidney, abscess of, blood in, 559; urine in, 325  
 acute nephritis, blood in, 646; urine in, 317  
 amyloid degeneration of, urine in, 322  
 arteriosclerosis of, urine in, 321  
 atrophy of, urine in, 324  
 cancer of, 325  
 chronic passive congestion of, urine in, 316  
 cloudy swelling of, urine in, 316  
 congenital cystic, blood in, 648; urine in, 325  
 diseases of, urine in, 315  
 fatty, urine in, 316  
 infarction of, 316  
 large white, urine in, 319  
 nephritis, blood in, 646 urine in, 317  
 parasitic diseases of, 327  
 senile atrophy of, urine in, 324  
 stone in, 327  
 tuberculosis of, 325  
 blood in, 627  
 Klebs-Löffler bacillus, 87  
 Köttdorfer's value, 409  
 Kulz and Vogel's test, pentose, 192

## L

Labor, albuminuria of women in, 234  
 Lactic acid, 694  
 in cancer of stomach, 393  
 in gastric juice, 371  
 quantitative determination of, 374  
 Lactose, 189  
 Laitose, 194  
 Lambia intestinalis in stools, 422  
 Landois method of determining blood alkalinity, 573  
 Large lymphocytes, 548  
 mononuclears, 535  
 white kidney, urine in, 319  
 Latent constipation, 399

Layer formation, sputum, 22  
 Lead in urine, 150  
 poison, cause of anæmia, 588  
 Lecithin, 596  
 globules in prostatic fluid, 313  
 Legal's test, 195  
 Leishman-Donovan bodies, 668  
 Leprosy, bacillus of, 54  
 blood in, 649  
 Leptodera intestinalis, 431  
 stercoralis, 431  
 Leptothrix innominata in sputum, 35  
 maximus buccalis, 36  
 Leucin, 693  
 as sediment, 263, 265  
 in sputum, 33  
 Leucoblast, 549  
 Leucocytes, 532, 535  
 counting, 472  
 fresh, 456  
 Leucocytosis, abscesses, 559  
 acute bronchitis, 558  
 acute cerebrospinal meningitis, 556  
 acute fibrinous pleurisy, 558  
 acute follicular tonsillitis, 556  
 acute ulcerative endocarditis, 557  
 agonal, 561  
 bronchiectasis, 558  
 cholera, 557  
 chronic bronchitis, 558  
 dementia præcox, 559  
 diabetic coma, 559  
 digestion, 553  
 empyema, 558  
 endocarditis, 557  
 erysipelas, 557  
 exercise, 561  
 fetid bronchitis, 558  
 gangrene of lungs, 559  
 gout, 559  
 hydronephrosis, 559  
 inflammations and febrile diseases, 555  
 influenza, 558  
 intestinal obstruction, 557  
 malignant disease, 560  
 Mastzell, 562  
 medicinal, 561  
 meningitis, 556  
 mixed, 562  
 myxœdema, 557  
 of eosinophiles, 564  
 of large mononuclears, 562  
 of new-born, 555  
 perirenal abscess, 559  
 pleurisy with effusion, 558  
 post-hemorrhagic, 560  
 post-operative, 566  
 pregnancy, 564  
 pyelitis, 559  
 pyelonephrosis, 559  
 pyogenic inflammations, 557, 558  
 rabies, 557  
 renal calculus, 559  
 uræmia, 559  
 whooping-cough, 556  
 Leucopenia, 563  
 Leucobillin, 405  
 Leukæmia, acute, 614

Leukæmia, lymphatic, 605, 611  
 mixed, 605  
 "parasite," 616  
 spleno-myelogenous, 605  
 Leukanæmia, 618  
 Levulose, 188  
 Levulosuria, 189  
 Leyden (Charcot-) crystals in blood, 609  
 in sputum, 34, 70  
 in stools, 414  
 Lieben's test, 196  
 Lientery, 412  
 Limit, assimilation for sugar, 169  
 Lipæmia, 637  
 Lipase, in stomach, 370  
 in urine, 168  
 Lipuria, 270  
 Litmus milk, 295  
 Liver abscess, blood in, 648  
 acute yellow atrophy, blood in, 650  
 cirrhosis, blood in, 648  
 diseases, blood in, 648  
 Lobar pneumonia, blood in, 629  
 chlorides in, 134  
 sputum of, 58  
 Lobulated cells, 538  
 Locomotor ataxia, spinal fluid in, 701  
 Löffler's blood serum, 87  
 methylene blue, 88, 290  
 Löwit's organism, 616  
 Löwy, alkalinity of blood, 574  
 Lues, blood in, 643  
 congenital, blood in, 635  
 diagnosis of, 681  
 organism of, 308  
 Luetic nephritis, 241, 319  
 Lung, abscess of, blood in, 559; sputum of, 80  
 actinomycosis of, sputum of, 42  
 cancer of, sputum of, 86  
 chronic passive congestion of, sputum of, 85  
 echinococcus of, 43  
 fluke, 44  
 gangrene of, blood in, 559; sputum of, 79  
 glanders of, 67  
 liver perforation through, 81  
 lues of, 87  
 œdema of, 83  
 stones, 24  
 tuberculosis of, blood in, 624; sputum in, 46  
 Lymph glands, eosinophilia in diseases of, 565  
 Lymphæmia, 611  
 Lymphocytes, 535, 538, 548  
 Lymphocytosis, 562  
 Lymphoid cells of Wolff, 548  
 Lymphomatosis, alyphæmic, 564

## M

MacNeal's stain, 310  
 Macrocytes, 507  
 Macrogamete, 652



Macrophages, 459  
 Macroscopic constituents  
   sputum, 22  
 Macroscopy of stools, 414  
 Magnesium phosphate in  
   urine, 148, 257  
 Malaria, blood in, 618, 652  
   granules in red cells,  
     510  
   of children, 635  
 Malarial parasites, æstivo-  
   autumnal, 657,  
     664  
   quartan, 655, 664  
   tertian, 652, 662  
 Malassez's hæmocyto-meter,  
   470  
 Malignant diseases, blood  
   in, 639  
   eosinophilia in,  
     566  
   gastric juice in,  
     363  
   leucocytes in, 560  
   of lung, sputum  
     of, 86  
 Maltose, 194  
 Maly method (Helmer),  
   375  
 Maragliano's endoglobular  
   degeneration, 454  
 Marrow, bone, 541  
   cells of Troje, 548  
 Masturbators, albuminuria  
   of, 235  
 Mastzell granules, 532  
   leucocytosis, 562  
 Mastzellen, 536, 548  
 Mature nucleated reds,  
   542  
 Meals, test, 353  
   Boas evening, 381  
   Ewald-Boas, 353  
   Fischer's, 354  
   lactic acid, free,  
     373  
   Riegel's, 353  
 Measles, blood in, 622  
   German, blood in, 622  
 Media for bacteria, 293  
 Mediastinal growths, 82  
 Medicinal eosinophilia, 566  
   leucocytosis, 561  
 Medicines, color of urine  
   due to, 106  
 Megaloblasts, 543  
 Megalocytes, 507  
 Megalogastric, 380  
 Megalokaryocyte, 550  
 Melanin, 104  
   as urine sediment,  
     263  
 Melanogen, 104  
 Mellituria, 194  
 Mellitus, diabetes, 203  
 Melting point of crystals,  
   determination of, 178  
 Membranous enteritis, 413  
   ureteritis, 227  
 Meningitis, leucocytes in,  
   556  
   tuberculous, blood in,  
     627  
   spinal fluid in, 701  
 Meningococcus, 701  
 Merozoite, 652  
 Metalbumin, 706  
 Methæmoglobin, 251  
 Methylene-blue degenera-  
   tion of Ehr-  
   lich, 510  
   stains, 88, 445  
   test in urine, 338  
   urine, 106

Methyl-violet test, 356  
 Methylxanthin, 126  
 Metrocytes, 547, 552  
 Mett's method for pepsin  
   determination, 367  
 Microblasts, 545  
 Micrococcus aureus, 291  
   catarrhalis, in spu-  
     tum, 34  
   intracellularis menin-  
     gitidis, 701  
   tetrageus in sputum,  
     36  
 Microcytes, 507  
 Microgamete, 652  
 Microgametocyte, 652  
 Microscopic constituents of  
   sputum, 26  
   examination of gas-  
     tric contents, 377  
 Microscopy of stools, 413  
 Miescher's hæmoglobinome-  
   ter, 435  
 Miliary tuberculosis, spu-  
   tum of, 46  
 Milk curds in stools, 413  
   litmus, 294  
 Mineral acidity of urine,  
   111  
 Minimal albuminuria, 228  
 Mixed leucocytosis, 528  
 Monocercomonas hominis,  
   390  
 Monont, 618  
 Mononuclears, eosinophile,  
   503, 514  
   large, 501  
     increase of, 528  
   neutrophile, 502, 513  
   small, 501, 504, 514  
 Moore's test, glucose, 173  
 Mörner's body, 225, 228  
   method, urea, 118  
 Morning sputum, 18  
   star crystals in urine,  
     255  
 Mosquito-cycle of malarial  
   parasite, 659  
 Motility of intestine, 399  
   of stomach, 380  
   determination of,  
     381  
   ectasis, 380  
   hypermotility, 380  
   motor insufficien-  
     cy, 380  
   salicylic-acid test,  
     381  
 Motor insufficiency, 380  
 Moulds in gastric contents,  
   378  
   in sputum, 24  
   in stools, 435  
   in urine, 310  
 Mouth flora, 92  
 Mucin in body fluids, 225,  
   692  
 Mucinuria, 227  
 Mucoid in body fluids, 227,  
   692  
   sputum, 19  
 Mucopurulent sputum, 19  
 Mucor corymbifer, 37  
   mucedo, 37  
   pusillus, 38  
   racemosus, 38  
   rhizopodiformis, 38  
   septatus, 38  
 Mucosa, atrophy of, 388  
   fragments of, in gas-  
     tric contents, 377  
 Mucous colitis, 410  
   threads in urine, 277

Mucus in stomach, 354,  
   364  
   in stools, 388  
   in vomitus, 351  
   sediment, 271  
 Muller's blood dust, 458  
 Murexid test, 124  
 Muscle fibres in gastric  
   contents, 377  
   in stools, 412  
 Mykæmia, 605  
 Myelin in sputum, 28  
 Myeloblast, 548  
 Myelocytes, eosinophile,  
   537, 548  
   neutrophile, 536, 547  
 Myelogenous leukaemia,  
   536, 547, 605  
 Myelopathic albumosuria,  
   241  
 Myxœdema, blood in, 650  
   leucocytes in, 557

# N

Nægeli's myeloblast, 548  
 Nakayama's test for bile,  
   160  
 Necrotic tissue fragments  
   in sputum, 22  
 Nelner's stain, 88  
 Nematode worms in urine,  
   311  
 Nephritis, acute, 317  
   albuminuria of, 240  
   blood in, 646  
   chronic diffuse, 319  
   indurative, 320  
   interstitial, 321  
   parenchymatous,  
     319  
   desquamative, 317  
   diffuse, 317  
   parenchymatous;  
     317  
   hæmoglobinurica, 318  
   hemorrhagic, 246  
   indurative, 320  
   lætic, 319  
   non-indurative, 319  
   subacute, 319  
   suppurative, 325  
   unilateral, 324  
   urine cultures in,  
     299  
 Nervous disease, blood in,  
   636  
   dyspepsia, 385  
   form of albuminuria,  
     240  
   system, sympathetic  
     diseases of, eosino-  
     philia in, 567  
 Neusser's granules, 534  
 Neutral sulphates, 146  
 Neutrophile granules, 534  
 Neutrophiles, small, 537  
 New-born, albuminuria of,  
   234  
   leucocytes in, 555  
 New growth in kidney,  
   urine in, 325  
   in mediastinum,  
     sputum in, 86  
 Night and day urine, 106  
 Nitric acid in urine, 147  
   test for albumin,  
     216  
   for urea, 122  
 Nitrogen of sputum, 19  
   of urine, 113  
   determination of,  
     115

Nitrogenous balance, 113  
 equilibrium, 113  
 Nitrous acid, 148  
 Non-indurative nephritis, 319  
 Noguchi test, 688  
 Normal persons, sputum of, 17  
 Normoblasts, 542  
   in pernicious anæmia, 593  
 Nubecula, 107  
 Nucleated reds, 542, 593  
   Howell's mature and immature, 542  
 Nuclei of reds, changes in, 545  
   fate of, 545  
 Nuclein bases, 126  
 Nucleo-albumin, 225, 227  
 Nucleohiston, 229  
 Nucleoid, 506  
 Number of red cells, 513  
 Nummular sputum, 58  
 Nutrition, effect of, on red count, 515  
 Nycturia, 96  
 Nylander's test, glucose, 175

**O**

Obermayer's test for indoxyl, 152  
 Occult hemorrhage in, stools, 378  
 Ochrosis, 107  
 Odor of sputum, 22  
   of urine, 107  
 Oedema, chloride retention in, 135  
 Oedema of lungs, sputum of, 82  
 Oesophagus, cancer of, blood in, 544  
*Oldium albicans* in sputum, 42  
   in stools, 437  
   coccidioides, 41  
   in blood, 588  
 Oligæmia, 576  
 Oligochromæmia, 530, 576  
 Oligocythæmia, 513, 576  
 Oligoplasma, 576  
 Oliguria, 97  
 Oliver's hæmocytometer, 470  
   hæmoglobinometer, 527  
 Oocyst, 652  
 Ookinet, 652  
 Operation, leucocytosis after, 559  
 Oppler-Boas bacillus, 399  
 Opsonic index, 676  
 Opsonins, 672  
 Orcin test, 192  
 Organic acids in gastric contents, 375  
 Organic acidity of urine, 112  
 Organized sediments, 271  
 Origin of casts, 279  
   of red cells, 546  
 Orthochromatic cells, 543  
 Orthostatic albuminuria, 234, 236  
 Orthotic albuminuria, 234  
 Osteoclasts, 550  
 Ovarian cysts, fluid of, 707  
 Oxalate, calcium, crystals in sputum, 34  
   in urine, 260

Oxalate, calcium, stones, 287  
 Oxalic acid, 261  
 Oxaluria, 259  
 Oxybutyric acid, 202  
 Oxyphilic granules, 532  
 Oxyproteinic acid, 132  
 Oxyuris vermicularis, 427

## P

Page's method for finding bacilli in solid stools, 437  
 Palpation, albuminuria due to, 239  
 Pancreatic disease, stools in, 442  
   fluid, 399  
   digestive power of, 400  
   secretion in vomitus, 339  
   stones, 416  
 Pancreon test, 442  
 Paper, Congo red, 356  
 Pappenheim's corallin stain, 52  
 Paracellair albuminurie, 239  
 Paracresol, 155  
 Paragonimus westermani, 44  
 Paramæcium coli, 424  
 Paramucin, 707  
 Parasites, anæmia due to, 587  
   animal, in lung, 43  
   in urine, 310  
   eosinophilia in, 565  
   in vomitus, 353  
   intestinal, 417  
   plant, in sputum, 35  
   in stools, 435  
 Parasitic hæmoptysis, 44  
 Paratyphoid bacillus, 295  
 Paraxanthin, 126  
 Parenchymatous nephritis, acute, urine in, 317  
   chronic, urine in, 319  
 Paresis, blood in, 636  
 Parovarian cyst fluid, 709  
 Paroxysmal hæmoglobinuria, 248  
   polyuria, 98  
 Pathological amounts of urine, 97  
   variations in red count, 517  
 Pavement-epithelial cells in sputum, 27  
 Pavy's disease, 234  
 Penicillium glaucum, 40  
   nummula, 40  
 Pentoses, 190  
 Pentosuria, 190  
 Penzoldt's test, 379  
 Pepsin, 364  
   quantitative method, 367  
   in urine, 168  
 Peptonuria, 243  
 Perforating empyema, lung, 82  
   pleurisy, serous, 82  
 Pericardial fluid, 706  
 Perinuclear granules of Neusser, 543  
 Periodic polyuria, 98

Perirenal abscess, leucocytes in, 559  
 Peritoneal fluid, analysis of, 703  
 Peritonitis, tuberculous, blood in, 627  
 Permeability, renal, 338  
 Pernicious anæmia, 589  
   gastric juice in, 363  
 Pertussis, leucocytes in, 556  
 Pettinkofer's reaction, 162  
 Phagocytic cells in leukaemia, 609  
 Phagocytosis, 672  
 Pharyngomycosis, 36  
 Phenol in urine, 151  
 Phenolsulphonaphthalein test, 342  
 Phenolsulphuric acid, 155  
 Phenylhydrazin test for glucose, 177  
 Phlegmonous gastritis, 386  
 Phlorizin test, 341  
 Phosphate concretions, 287  
   crystals in sputum, 34  
 Phosphates, calcium, 258  
   in urine, 137  
   magnesium, 258  
   triple, 257  
 Phosphaturia, 109  
 Phosphaturique albuminurie, 238  
 Phosphorus-containing proteids, 692  
 Phosphorus poisoning, red count, 517  
   hæmoptysical, 58  
 Phthisis, blood in, 624  
   melanotica, 28  
   stone-cutters, 71  
 Physiological albuminuria, 234  
   eosinophilia, 564  
   variation in red count, 514  
 Physiology of gastric secretion, 362  
 Piffard's method for staining bacteria, 291  
 Pigment, bile, in sputum, 20  
   in urine, 155  
   blood, in sputum, 20  
   coal, in sputum, 21, 28  
   of urine, 150  
 Pigmented cells in blood, 550  
   in sputum, 28  
 Piroplasmosis, 658  
 Pittsfield's method, 292  
 Placental cells, 459  
 Plant parasites in sputum, 35  
   in stools, 435  
   in urine, 312  
 Plasmodium præcox, 657  
   vivax, 652  
 Plastic bronchitis, sputum of, 76  
 Platelets of blood, 568  
 Plaut's angina, 92  
 Plehn's granules, 510  
 Plethora vera, 576  
 Pleural fluid, analysis of, 703  
   cytodiagnosis of, 703  
 Pleurisy, acute fibrinous, leucocytes in, 558  
   serous, perforating, sputum in, 82

Pleurisy, with effusion, leucocytes in, 558  
 Plugs, Dittrich's, 23  
     prostatic, 324  
 Pneumaturia, 310  
 Pneumokoniosis, 21, 87  
 Pneumoliths, 25  
 Pneumonia, abscess of lung, 82  
     blood in, 629  
     broncho-, blood in, 631  
     sputum of, 64  
     chlorides in, 134  
     chronic, sputum in, 64  
     croupous, sputum in, 58  
     desquamatory catarrhal, 28  
     hemorrhage, sputum in, 60  
     interstitial, 64  
     sputum of, 58  
     subacute, sputum in, 65  
 Pneumonomycosis aspergillina, 39  
 Poikilocytes, 506, 593  
 Poisons, anæmia due to, 588  
 Polar granules, 38  
 Polariscope, 184  
 Polychromatophilia, 509, 593  
     partial, 510  
 Polychrome methylene-blue stains, 481  
 Polycyclic elimination, 338  
 Polycythæmia, 513  
 Polymorphonuclear leucocytes, 536  
 Polyplasmia, 576  
 Polyuria, epieritral, 97  
     paroxysmal, 98  
     periodic, 98  
 Poor, anæmia of the, 583  
     food, anæmia due to, 583  
 Post-febrile eosinophilia, 566  
 Post-hemorrhagic anæmia, 579  
 Post-infectious albuminuria, 238  
 Postural albuminuria, 234  
     236  
 Potassium ferrocyanide test for albumin, 218  
     in urine, 149  
     iodide test, 340  
 Precipitins, 681  
 Pregnancy, ammonia in, 127  
     leucocytosis of, 554  
     red cells in, 515  
 Pregnant women, albuminuria of, 234  
 Prégoutteuse albuminurie, 238  
 Preservation of sediments, 252  
     of urine, 94  
 Primary anæmia, 577, 589  
     pernicious anæmia, 589  
 Productiva, pyelitis, 227  
 Products of gastric digestion, 371  
 Progressive pernicious anæmia, 577, 589  
 Proliferating cysts, Pflüger's tubules, 708  
 Prostatic casts, 314  
     fluid, 312

Prostatic plugs, 306  
 Prostatitis, 307  
 Prostatorrhœa, 307  
 Proteids in body fluids, 692  
     of urine, 223  
 Proteus bacillus, 296  
     cystitis, 301  
 Protocleucocytes, 548  
 Protoryxomyces coprinarius, 422  
 Prune-juice sputum, 60  
 Pseudo-casts, 278  
 Pseudodiphtheria bacilli, 88  
 Pseudo-elastic tissue, 31  
 Pseudo-gall-stones, 415  
 Pseudoglobulin, 224  
 Pseudoleucocytosis, 560  
 Pseudoleukæmia, 617  
     of children, 635  
 Pseudolymphocytes, 537  
 Pseudomucin, 707  
 Pseudothipsis calcuosa, 25  
 Puberty, albuminuria, of, 234  
 Pulmonary actinomycosis, 42  
     cancer, 86  
     echinococcus disease, 43  
     gangrene, 79  
     infarction, 85  
     lues, 87  
     tuberculosis, blood in, 624  
     sputum in, 46  
 Purdy's fluid, 182  
 Purin bases, 126  
 Purulent gastritis, 386  
 Pus casts, 276  
     cells in prostatic fluid, 313  
     formation, anæmia due to, 586  
     in gastric juice, 377  
     in sputum, 27  
     in stools, 412  
     in urine, 284  
     masses in sputum, 22  
 Putrid bronchitis, sputum of, 75  
 Pycnometer, 482  
 Pyelitis, cultures in, 300  
     leucocytes in, 559  
     urine in, 316  
 Pyelitis productiva, 221  
 Pyelonephritis, 316  
 Pyelonephrosis, leucocytes in, 529  
 Pyloric stenosis, 381  
 Pyocyanin, 290  
 Pyogenic albumosuria, 239  
     inflammations, leucocytes in, 527, 528  
 Pyonephrosis, urine in, 317  
 Pyrocatechin, 101, 151

Q

Quadrurates, 120, 248  
 Quartan malaria, parasite of, 627, 636  
 Quotient, albumin, 217

R

Rabies, blood in, 527  
     of sputum, 19  
     of stools, 403  
     of urine, 103

Rectum, cancer of, blood in, 615  
     stools in, 424, 442  
 Red cells, 450, 505  
     color of, 453  
     counting methods, 459  
     crenated, 451  
     deformed, 451, 506  
     degenerations of, 454, 509  
     granules in, 456, 511  
     in sputum, 29  
     in urine, 246, 285  
     nucleated, 542  
     number of, 513  
     resistance of, 519  
     shape of, 451, 506  
     size of, 452, 507  
     structure of, 506  
     indigo, in urine, 154  
     pepper granules, 254  
 Reducible body of Stokvis, 158  
 Reichert-Meissl value, 409  
 Reichmann's disease, 383  
 Relapsing fever, 671  
 Renal abscess, urine in, 325  
     atrophy, 324  
     urine in, 324  
     calculus, 286, 327  
     leucocytes in, 559  
     concretions, 286  
     diagnosis, 328  
     disease, albuminuria, 240  
     blood in, 646  
     urine in, 315  
     epistaxis, 246  
     epithelial cells in urine, 271  
     hæmaturia, 246  
     hæmophilia, 247  
     permeability, 338  
 Rennet, 371  
 Résiduale albuminurie, 328  
 Residue of dried blood, 485  
 Resistance of red blood, 519  
 Retention of chlorides, 135  
 Rhabditis stercoralis, 321  
     strongyloides, 321  
 Rheumatism, acute articular, blood in, 633  
 Rice-water vomitus, 352  
 Rickets, blood in, 636  
 Riegel's meal, 353  
 Ring body of Cabot, 510  
 Roberts method, quantity of albumin, 222  
     quantity of glucose, 187  
 Rosaniline test, 340  
 Rosenau's blood agar, 294  
 Rosenbach's reaction, 162  
     test, bile, 159  
 Rot, grinder's, sputum of, 21  
 Rubner's test, glucose, 180  
     lactose, 190  
 Rusty sputum, 20

S

Saccharomyces busse, 36  
 Saccharomycosis, 36  
 Sago granules in sputum, 28  
 Sahli's hæmometer, 526

- Salicylic acid test for gastric motility, 381  
 of renal sufficiency, 340
- Salkowski's method of determining the alkalinity of blood, 575  
 test for pentose, 191
- Sand, gall, 415  
 intestinal, 416
- Saprophytes in urine, 312
- Sarcinæ in gastric contents, 378, 395  
 in stools, 435  
 in urine, 310
- Sarcoma, blood in, 643
- Saturation deficit, 359
- Scarlet fever, blood in, 623
- Scheme for sediments, 267
- Schistosoma hæmatobium in stools, 435  
 in urine, 311
- Schizogone, 652
- Schizont, 652
- Schlesinger's test, 406
- Schlössing method for ammonia determination, 128
- Schmalz tubes, 482
- Schmidt's method bilirubin in stools, 405
- Schönbein's test for blood, 250
- Schöndorff method, urea determination, 118
- Schöttli's enriching method, 439
- Schultze's granular masses, 568
- Sclerosis (arterio-) of kidney, urine in, 321
- Scurvy, blood in, 650
- Scybala, 403
- Secondary anæmia, 577
- Secretion, continuous, of gastric juice, 383  
 gastric, physiology of, 362  
 of water by stomach, 380
- Sedimentation of red blood-corpuscles, 485
- Sediments, urine, 252  
 bilirubin, 262  
 carbonates, 257  
 cholesterin, 263  
 cystin, 266  
 hæmatoidin, 262  
 hæmoglobin, 263  
 heteroalbumose, 262  
 hippuric acid, 262  
 indigo, 263  
 leucin, 263, 265  
 melanin, 263  
 mucous, 271  
 organized, 271  
 oxalates, 259  
 phosphates, 257  
 preservation, 252  
 scheme, 267  
 tyrosin, 263, 265  
 unorganized, 254  
 urates, 254  
 xanthin, 262
- Senile atrophy of kidney, urine in, 324
- Septicæmia, blood in, 621  
 urine in, 296
- "Seromucus sputum," 71
- Serous cysts, ovarian, 707  
 pleurisy perforating through lung, 82
- Serous sputum, 19  
 of oedema of lung, 82
- Serum albumin in urine, 223  
 diagnosis, value of, 505  
 hæmolytic, 684  
 globulin in urine, 224
- Seven-glass test, 307
- Sex, effect on count of red cells, 514
- Shape of red cells, 506
- Shiga's bacillus, 442
- Showers of casts, 281
- Siderosis, 21, 87
- Silicic acid in urine, 147
- Size of casts, 278  
 of red cells, 507
- Sjöqvist's method of urea determination, 118
- Skatoxyl, 153
- Skin, eosinophilia in diseases of, 565
- Small mononuclears, 535, 538, 548  
 neutrophiles, 537
- Smallpox blood in, 623
- Smears, blood, 474
- Smegma bacillus, 54, 292  
 bacilli in urine, 292
- Soaps in stools, 409  
 determination, 409
- Sodium in urine, 149
- Soft chancre, organism of, 308
- Specific gravity of blood, 482  
 of body fluids, 692  
 of urine, 99  
 method of determining glucose, 187
- Spectroscope, 251
- Spermatocele, 710
- Spermatorrhœa, 307
- Spermatozoa, 313, 314
- Spermin crystals in prostatic fluid, 314
- Spiegler's albumin reagent, 219
- Spinal fluid, 696  
 in general paresis, 701  
 in tabes dorsalis, 701  
 in tuberculous meningitis, 701
- Spirals, Curschmann's, 23, 68
- Spirillum carteri, 671  
 cholerae asiaticæ, 438  
 of Deneke, 439  
 duttoni, 671  
 of Massea, 439  
 of Metchnikoff, 439  
 Novyi, 671  
 obermeieri, 671
- Schuylikillensis, 439
- Spirochæte of mouth, 92  
 of Obermeier, 671
- Spirochæte pallidum, 308  
 refringens, 310
- Spleen, eosinophilia in diseases of, 564
- Splenic anæmia, 589
- Splenomegaly, red cells in, 518
- Spore stains, 291
- Sporoblast, 652
- Sporogone, 652
- Sporozoite, 652
- Sputum, 17  
 air in, 22
- Sputum, amount, 18  
 animal parasites of, 43  
 bacteria in, 59  
 bile-stained, 20  
 blood in, 29  
 bloody, 20  
 character of, 19  
 chemical analysis of, 45  
 children's, 18  
 color of, 19  
 consistency of, 19  
 crystals, 33  
 foreign bodies in, 20  
 green, 20, 60  
 hæmoglobin stained, 20  
 macroscopic, 22  
 microscopic, 26  
 morning, 18  
 mucoid, 19  
 mucopurulent, 19  
 nitrogen of, 19  
 normal persons, 17  
 odor of, 22  
 plant parasites, 35  
 prune-juice, 60  
 purulent, 19  
 reaction of, 19  
 rusty, 20  
 sarcinæ in, 36  
 serous, 19  
 yeasts in, 36
- Sputum coctum, 70  
 crudum, 69  
 globosum, 58
- Staining casts, 282  
 properties of cells, 509  
 vital, 510
- Stains for bacteria, 290  
 for blood, 490
- Staphylococcus epidermidis albus, 298  
 pyogenes albus, 297  
 pyogenes aureus, 297
- Starch, digestion of, 371
- Staubli's method, 671
- Stippled cells, 511
- Stokvis reducible body, 158  
 test, bile, 160
- Stollinow's method of quantitative albumin, 222
- Stomach, absorption power of, 379  
 aseptic condition of, 352  
 bacteria in, 378  
 cancer of, blood in, 641  
 contents, 350  
 dilated, 381  
 fasting, 353  
 motility of, 380  
 sarcinæ in, 378  
 ulcer of, 389
- Stomatomycosis, 36
- Stone in bladder, 286  
 in kidney, 286, 327  
 in lung, 24  
 in ureter, 327
- Stone-cutters' phthisis, 21
- Stones, gall, 414  
 intestinal, 416  
 pancreatic, 416  
 pseudo-gall, 415
- Stools, alcoholic, 404, 406  
 albumin in, 412  
 bacteria in, 412  
 bile pigments in, 405  
 blood in, 410  
 carbohydrates in, 412  
 clay-colored, 404

Stools, color of, 404  
 concretions in, 415  
 consistency of, 402  
 constituents of, 402  
 crystals in, 413  
 epithelium cells in, 413  
 examination of, 402  
 fatty, 406  
 ferments in, 413  
 forms of, 403  
 frequency of, 403  
 in disease, 437  
 in pancreatic disease, 442  
 macroscopy, 414  
 microscopy, 413  
 mucus in, 409  
 muscle-fibres in, 349  
 parasites in, 417  
 method of preserving, 445  
 reaction of, 403  
 starch in, 412  
 Strassburger's bile-acid test, 163  
 Strauss' test, lactic acid, 373  
 Streptococcus cystitis, 301  
 mucosus capsulatus, 35  
 pyogenes, 298  
 Streptothrix pseudotuberculosis, 35  
 Strongyloides intestinalis, 431  
 anæmia due to, 481  
 Structural albuminuria, 231  
 Structure of red cells, 506  
 Subacidity in cancer of stomach, 392  
 Subacute indurative pneumonia, sputum in, 65  
 nephritis, urine in, 319  
 Succinic acid, 693  
 Sulphæmoglobinæ mia, 495  
 Sulphates of urine, 142  
 easily split, 147  
 ethereal, 142, 145  
 neutral, 146  
 total, 142  
 unoxidized, 143  
 Sulphide of hydrogen in urine, 147  
 Sulphocyanic acid, 147  
 Sulphur, bile-acid test of Hay, 163  
 Superacidity, 382  
 Supersecretion, 383  
 Suppurative nephritis, urine in, 325  
 Surgical values of tests (renal functional), 347  
 Sympathetic nervous system, eosinophilia in diseases of, 567  
 Synovial fluid, 706  
 Syphilis, albuminuria of, 319  
 lung, 87  
 nephritis in, 319  
 organism of, 308

T

Tabes dorsalis, spinal fluid in, 701  
 Table for detection of calculi, 288

Tænia cricumerina, 434  
 nana, 433  
 saginata, 432  
 solium, 432  
 Tallquist scale, 528  
 Tanret's solution, 219  
 Teichmann's test, 250  
 Temperature, effect of, on count of reds, 515  
 Tertian malaria, 652, 662  
 Test meal, Boas evening, 381  
 breakfast, 353  
 lactic acid free, 373  
 Ewald-Boas, 353  
 Fischer, 354  
 for intestinal examination, 400  
 Riegel, 353  
 Testicle, cancer of, blood in, 643  
 Testicular casts in urine, 283  
 Tetanus, bacillus of, 297  
 Tetrigenus, micrococcus, in sputum, 36  
 Therapeutic measures, effect of, on count of reds, 517  
 Thionin stain, 482  
 Thiosulphuric acid, 147  
 Thoma-Zeiss blood counter, 459  
 Thoracentesis, cause of albuminous expectoration, 83  
 Thornapple crystals, 255  
 Threads, mucous, in urine, 277  
 Throat cultures, 87  
 Tide, alkaline, 109  
 Tissue, elastic, in sputum, 29, 48  
 Tissue fragments in sputum, 22  
 in urine, 284  
 lung, in gangrene, 80  
 pseudo-elastic, in sputum, 31  
 Toisson's fluid, 460  
 Tonsillitis, leucocytes in, 556  
 Töpfer's method, 360  
 Tophus, 710  
 Total acidity, gastric, 360  
 of urine, 111  
 organic acids in gastric juice, 375  
 Toxæmic jaundice, 156  
 Transitional leucocytes, 535  
 Transparent cells, 538  
 Transudates, 702  
 Traumatic albuminuria, 239  
 Trematode worms as parasites, 432  
 Trichina (Trichinella) spiralis, 425  
 in blood, 671  
 Trichiuris trichiura, 430  
 Trichocephalus dispar, 430  
 Trichomonas hominis, 422  
 intestinalis, 390  
 pulmonalis, 43  
 vaginalis, 422  
 in urine, 310  
 Tripanoma pallidum, 308  
 Triple phosphate, 257  
 concretions, 307  
 in sputum, 33  
 Tripperfiliden in urine, 306  
 Troje's marrow cell, 548

Trommer's test, glucose, 172  
 Tropælin OO, 354  
 Tropics, anæmia of, 585  
 Trousseau's test, bile, 160  
 True albuminuria, 230  
 Trypanosomiasis, 666  
 Trypsin, 399  
 in stools, 413  
 in urine, 168  
 Tryptophan test, 396  
 Tubercle bacilli, in cerebro-spinal fluid, 701  
 in sputum, 49  
 in stools, 436  
 in urine, 292  
 Tubercle bacillus, 49  
 isolation, 49  
 prognostic value, 56  
 staining, 50  
 Tuberculosis, blood in, 624  
 acute miliary, blood in, 627  
 adrenals, blood in, 627  
 of bladder, 300  
 bones and joints, blood in, 627  
 chronic, of lungs, blood in, 625  
 intestine, 627  
 kidneys, blood in, 627  
 urine in, 325  
 lymph glands, blood in, 618  
 meningitis, blood in, 627  
 peritonitis, blood in, 627  
 pulmonary, acute miliary, 46  
 bronchopneumonia, 47  
 chronic ulcerative, 47  
 fibroid form, 46  
 hæmophysical, 55  
 sputum of, 46  
 Tuberculous meningitis, 701  
 Tubo-ovarian cysts, 709  
 Tubular insufficiency, 337  
 Tumor fragments in gastric juice, 395  
 in stools, 417  
 in urine, 284  
 Typhoid bacillus, 295  
 in stools, 437  
 Typhoid fever, blood in, 627  
 stools in, 437  
 Typhus fever, blood in, 622  
 Tyrosin, 693  
 in sputum, 33  
 in urine, 263, 265

U

Udransky's test, bile acid, 163  
 Uffelmann's test, lactic acid, 373  
 Ulcer of duodenum, 390  
 of stomach, 389  
 Ulceration, chronic tuberculous, sputum in, 47  
 Uleïnaria americana, 427  
 duodenalis, 427  
 Uncinariasis, anæmia in, 587  
 Unilateral nephritis, 324  
 Unorganized sediment, 254  
 Unoxidized sulphur in urine, 143

Stools, color of, 404  
 concretions in, 413  
 consistency of, 402  
 constituents of, 402  
 crystals in, 413  
 epithelium cells in, 413  
 examination of, 402  
 fatty, 406  
 ferments in, 413  
 forms of, 403  
 frequency of, 403  
 in disease, 437  
 in pancreatic disease, 442  
 macroscopy, 414  
 microscopy, 413  
 mucus in, 409  
 muscle-fibres in, 349  
 parasites in, 417  
 method of preserving, 445  
 reaction of, 403  
 starch in, 412  
 Strassburger's bile-acid test, 163  
 Strauss' test, lactic acid, 373  
 Streptococcus cystitis, 301  
 mucosus capsulatus, 35  
 pyogenes, 298  
 Streptothrix pseudotuberculosis, 35  
 Strongyloides intestinalis, 431  
 anæmia due to, 481  
 Structural albuminuria, 231  
 Structure of red cells, 506  
 Subacidity in cancer of stomach, 392  
 Subacute indurative pneumonia, sputum in, 65  
 nephritis, urine in, 319  
 Succinic acid, 693  
 Sulphæmoglobinæ mia, 495  
 Sulphates of urine, 142  
 easily split, 147  
 ethereal, 142, 145  
 neutral, 146  
 total, 142  
 unoxidized, 143  
 Sulphide of hydrogen in urine, 147  
 Sulphocyanic acid, 147  
 Sulphur, bile-acid test of Hay, 163  
 Superacidity, 382  
 Supersecretion, 383  
 Suppurative nephritis, urine in, 325  
 Surgical values of tests (renal functional), 347  
 Sympathetic nervous system, eosinophilia in diseases of, 567  
 Synovial fluid, 706  
 Syphilis, albuminuria of, 319  
 lung, 87  
 nephritis in, 319  
 organism of, 308

## T

Tabes dorsalis, spinal fluid in, 701  
 Table for detection of calculi, 288

Tania cricumerina, 434  
 nana, 433  
 saginata, 432  
 solium, 432  
 Tallquist scale, 528  
 Tanret's solution, 219  
 Teichmann's test, 250  
 Temperature, effect of, on count of reds, 515  
 Tertian malaria, 652, 662  
 Test meal, Boas evening, 381  
 breakfast, 353  
 lactic acid free, 373  
 Ewald-Boas, 353  
 Fischer, 354  
 for intestinal examination, 400  
 Riegel, 353  
 Testicle, cancer of, blood in, 643  
 Testicular casts in urine, 283  
 Tetanus, bacillus of, 297  
 Tetragnus, micrococcus, in sputum, 36  
 Therapeutic measures, effect of, on count of reds, 517  
 Thionin stain, 482  
 Thiosulphuric acid, 147  
 Thoma-Zeiss blood counter, 459  
 Thoracentesis, cause of albuminous expectoration, 83  
 Thornapple crystals, 255  
 Threads, mucous, in urine, 277  
 Throat cultures, 87  
 Tide, alkaline, 109  
 Tissue, elastic, in sputum, 29, 48  
 Tissue fragments in sputum, 22  
 in urine, 284  
 lung, in gangrene, 80  
 pseudo-elastic, in sputum, 31  
 Toisson's fluid, 460  
 Tonsillitis, leucocytes in, 556  
 Töpfer's method, 360  
 Tophus, 710  
 Total acidity, gastric, 360  
 of urine, 111  
 organic acids in gastric juice, 375  
 Toxæmic jaundice, 156  
 Transitional leucocytes, 535  
 Transparent cells, 538  
 Transudates, 702  
 Traumatic albuminuria, 239  
 Trematode worms as parasites, 432  
 Trichina (Trichinella) spiralis, 425  
 in blood, 671  
 Trichiuris trichiura, 430  
 Trichocephalus dispar, 430  
 Trichomonas hominis, 422  
 intestinalis, 390  
 pulmonalis, 43  
 vaginalis, 422  
 in urine, 310  
 Tripanoma pallidum, 308  
 Triple phosphate, 257  
 concretions, 307  
 in sputum, 33  
 Tripperfäden in urine, 306  
 Troje's marrow cell, 548

Trommer's test, glucose, 172  
 Tropælin OO, 354  
 Tropics, anæmia of, 585  
 Trousseau's test, bile, 160  
 True albuminuria, 230  
 Trypanosomiasis, 666  
 Trypsin, 399  
 in stools, 413  
 in urine, 168  
 Tryptophan test, 396  
 Tubercle bacilli, in cerebro-spinal fluid, 701  
 in sputum, 49  
 in stools, 436  
 in urine, 292  
 Tubercle bacillus, 49  
 isolation, 49  
 prognostic value, 56  
 staining, 50  
 Tuberculosis, blood in, 624  
 acute, military, blood in, 627  
 adrenals, blood in, 627  
 of bladder, 300  
 bones and joints, blood in, 627  
 chronic, of lungs, blood in, 625  
 intestine, 627  
 kidneys, blood in, 627  
 urine in, 325  
 lymph glands, blood in, 618  
 meningitis, blood in, 627  
 peritonitis, blood in, 627  
 pulmonary, acute, military, 46  
 bronchopneumonia, 47  
 chronic, ulcerative, 47  
 fibroid form, 46  
 hæmophysical, 55  
 sputum of, 46  
 Tuberculous meningitis, 701  
 Tubo-ovarian cysts, 709  
 Tubular insufficiency, 337  
 Tumor fragments in gastric juice, 395  
 in stools, 417  
 in urine, 284  
 Typhoid bacillus, 295  
 in stools, 437  
 Typhoid fever, blood in, 627  
 stools in, 437  
 Typhus fever, blood in, 622  
 Tyrosin, 693  
 in sputum, 33  
 in urine, 263, 265

## U

Udransky's test, bile acid, 163  
 Uffelmann's test, lactic acid, 373  
 Uleer of duodenum, 390  
 of stomach, 389  
 Ulceration, chronic tuberculous, sputum in, 47  
 Ulcinaria americana, 427  
 duodenalis, 427  
 Uncinariasis, anæmia in, 587  
 Unilateral nephritis, 324  
 Unorganized sediment, 251  
 Unoxidized sulphur in urine, 143

